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CONTENTS

(Vol. XXV, Part IV)

(December 1955)

ORIGINAL ARTICLES

STUDIES ON THE DISEASES OF *MANGIFERA INDICA* LINN*

VIII. OCCURRENCE OF DEPOSITS IN NECROTIC MANGOES

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(Received for publication on 20th October 1953)

(With Plates XI-XII, 10 text-figures and two appendices)

WHILE investigating the internal changes associated with necrosis of the mango fruit, Das-Gupta and Sinha [1939, 1944] discovered the presence of brown deposits in the vessels of the outer mesocarp. They advanced the suggestion that the appearance of these deposits was the first histological sign of the disease. The presence and distribution of deposits in conducting channels of necrotic fruits belonging to six mango varieties were further investigated by Das-Gupta and Asthana and the results published in the form of a short note in 1944. But before the detailed paper was ready for publication some further data had accumulated, and it was thought advisable to make a more comprehensive study of the problem employing larger number of fresh and preserved mangoes in various stages of necrosis and belonging to a larger number of varieties. As a result of this study extending from 1944 to 1947, more extensive data accumulated bringing fresh facts to light. The present paper records in detail these observations, which supplement and to a certain extent modify the earlier observations.*

Material and Method

The material for investigations on the occurrence of deposit comprised 10 varieties of mango fruits, viz. Dasehri, Safeda, Tamburia, Langra, Bombai, Khajli, Kishanbhog, Husnara, Maldaha and Gola, collected from the various diseased and healthy orchards of Lucknow, Rampur, Fatehgarh, Mainpuri and Hardoi districts in Uttar Pradesh. The mangoes utilised were classed as healthy, unhealthy and diseased which are defined as follows:

Healthy. These are mangoes obtained from healthy orchards. These do not show any external symptoms of necrosis nor the presence of internal deposits. Healthy orchards are those where necrosis has never been reported.

Unhealthy. These are apparently healthy mangoes obtained from diseased orchards. The mangoes are free from external symptoms of necrosis. Diseased orchards are those where necrosis is known to occur.

Necrotic. These are mangoes which show even the slightest external symptom of necrosis.

(i) *Early stage.* When tip is aetiolated.

(ii) *Advanced stage.* When necrotic tissue in the tip has collapsed.

* Contribution from the Department of Botany, Lucknow University, New series No. 14. The delay in the publication of this paper is due to long absence of the senior author from India.

The healthy mangoes were collected from Horticulture Garden, and the unhealthy and necrotic mangoes from the Nawabali orchard, Lucknow. The fruits from the respective orchards were brought to laboratory immediately after plucking and some were examined fresh ; the others were preserved in formalin (4 per cent), alcohol (90 per cent) and form-acetic alcohol. The out-station mangoes were similarly preserved shortly after arrival. These were then examined after different intervals of preservation ranging from 24 hours to four years.

The methods employed were usual, hand and microtome sections, simple peeling, and mostly maceration. For peeling the outermost part of the mesocarp was removed by means of a sharp scalpel when deposits in conducting channels, if present, could be seen as brownish-red streaks in the flesh. This method was found to be very successful with fruits preserved in formalin and form-acetic alcohol. For maceration thin slices of outer and inner mesocarp, and small portions of entire mangoes cut in a series were boiled separately in 5-10 per cent potassium hydroxide solution till the parenchymatous tissue got disintegrated. The material was then slowly washed in a stream of running water to remove the destroyed tissue and all traces of alkali employed. The strands of ducts and vessels were dehydrated in alcohol, and mounted in canada balsam after clearing in xylol or in Euparal. Glycerine mounts were also made wherever necessary. From these preparations the location and distribution of deposits could be clearly made out. Deposits in alkali-treated mangoes were found to undergo a slight change of colour and, therefore, as far as possible, reference was made to deposits in untreated fresh mangoes for describing their colour.

In order to facilitate the description of the distribution of deposits in various parts of the fruit, mango for the present purpose has been divided into shoulder, trunk and tip with further sub-divisions. This division is at slight variance with the conventional method as given by Naik and Gangolly [1950] in that in the latter no trunk is recognised and the fruit as far as one could gather is divided into base, shoulder, sinus and apex. It is, however, a matter of detail as will be seen from the description and Fig. 1.

Upper shoulder . . .	Corresponds to the conventional *base* region.
Lower shoulder . . .	Extends from the lower limit of base to the line of maximum diameter.
Upper trunk . . .	Extends from lower limit of lower shoulder upto the upper limit of sinus.
Lower trunk . . .	Corresponds to the conventional sinus belt, extending upto the beak.
Tip	Corresponds to the apex, upper limit being the beak line.

The following brief description of the character and distribution of conducting channels in the mango fruit will facilitate the understanding of distribution of deposits found in them. In mango fruits the ducts when they emerge from stalk take two courses. One of them runs in the outer mesocarp, whereas the other in the innermost mesocarp. The outer mesocarp contains larger number of ducts (Fig. 2).

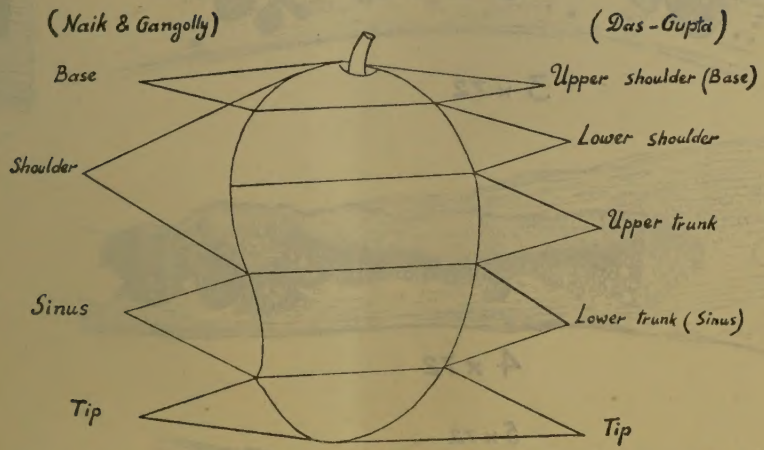


FIG. 1. A mango with different parts labelled according to the nomenclatures of Naik and Gangolly and Das-Gupta (XI).

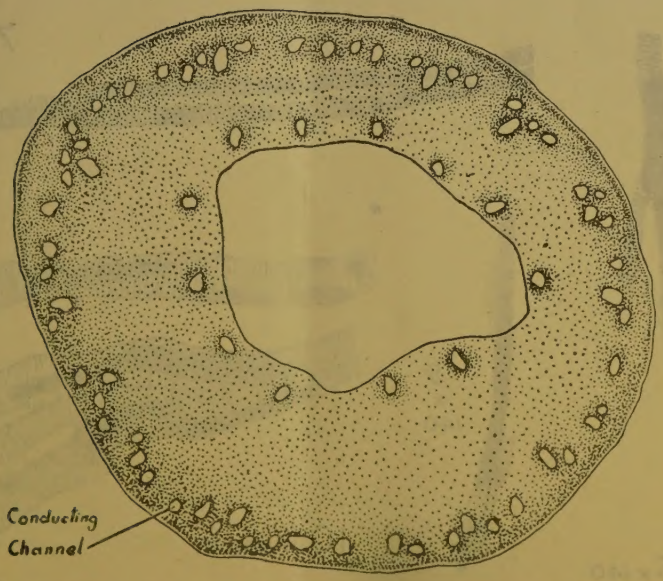


FIG. 2. Transverse section of mango showing outer mesocarp rich in conducting channels (X3.5).

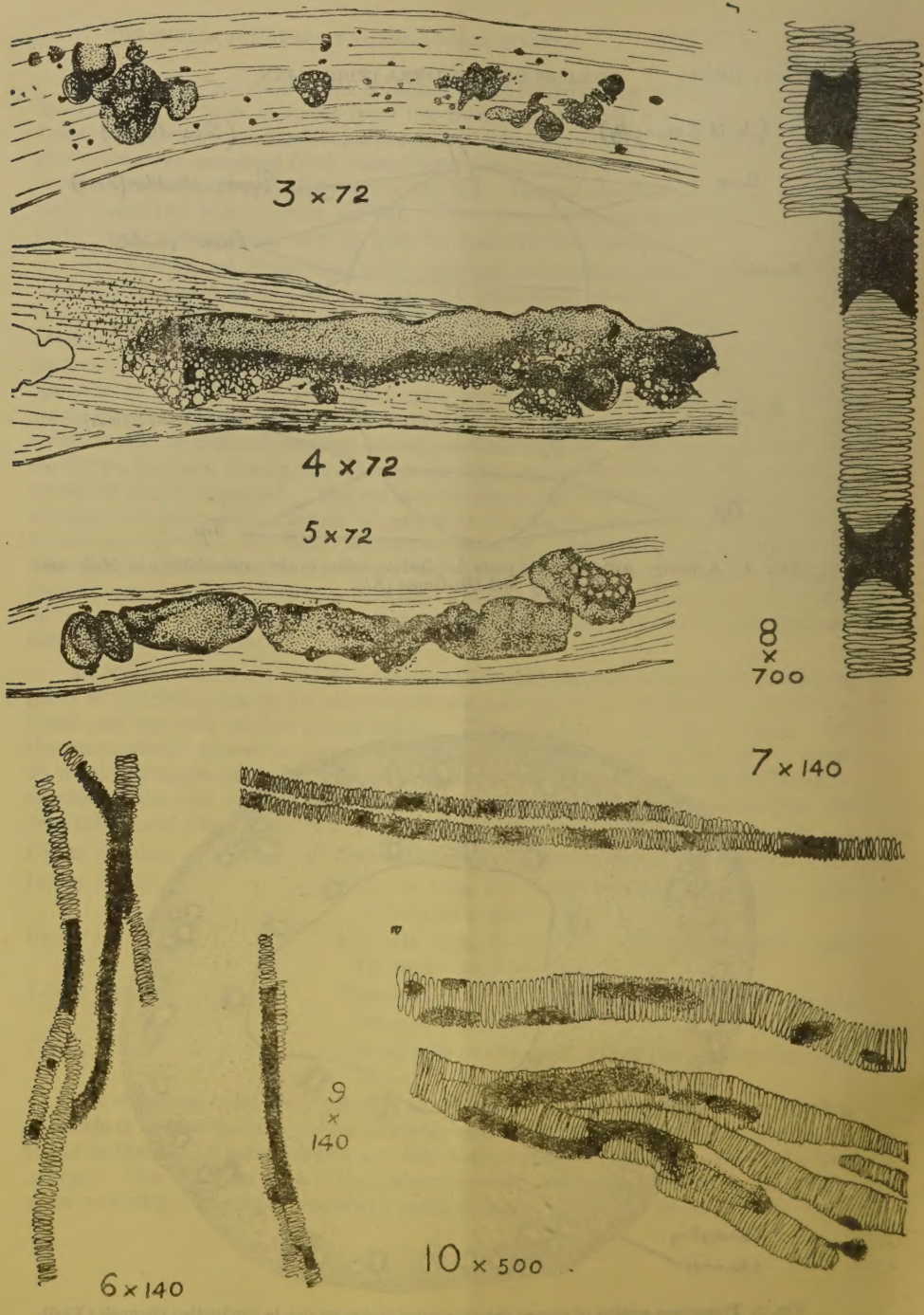


FIG. 3-5. Deposits in ducts under preservation: Fig. 3. Oily viscous fluid taking a globular form. Fig. 4-5. Solid deposits in ducts under preservation. Fig. 6-9. Deposits in vessels under fresh condition showing complete or partial choking of the lumen. Fig. 10. Deposits in vessels in various stages of aggregation.

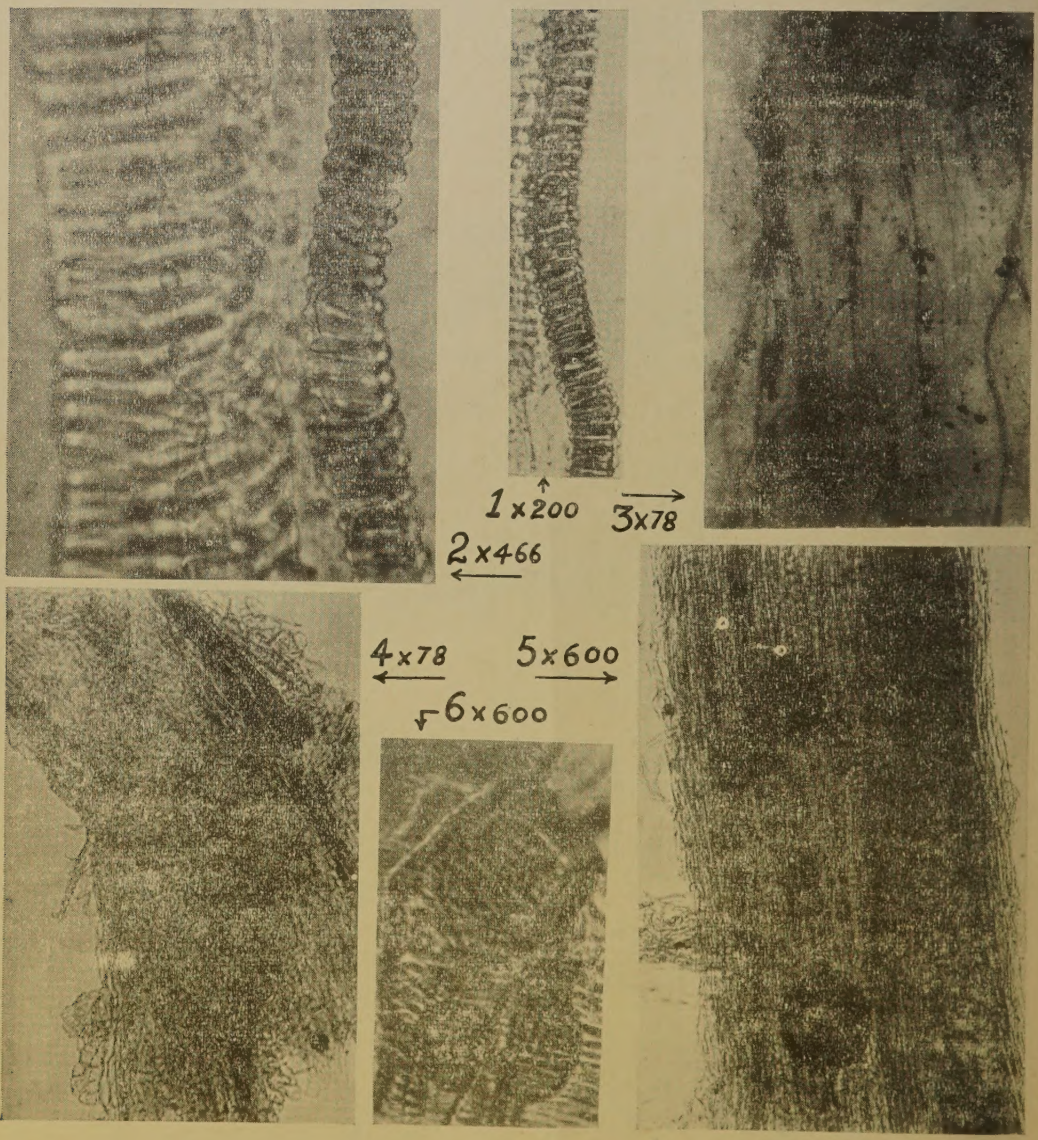


FIG. 1-6. Microphotographs showing deposits in conducting channels of necrotic mangoes (from macerated preparations). 1. Deposits choking the lumen of vessels in fresh conditions. 2. Part of Fig. 1 magnified. 3. Appearance of ducts of inner mesocarp of fresh necrotic mangoes. 4-6. Deposits in necrotic mangoes under preservation: (*a*) Vessels completely choked, (*b*) Oily deposits in ducts having resolved themselves into globules.

As has already been pointed out by Das-Gupta and Sinha [1944] the ducts are always accompanied by a vessel. In Dasehri variety the mesocarp is abundantly supplied by amphicribral vascular bundles consisting of protoxylem elements only. In the inner mesocarp the vascular bundle is very much reduced and the characteristic phloem is lacking, the function being performed by a few neighbouring parenchymatous cells. The endocarpic vascular supply, too, consists of protoxylem elements. It receives a stout vascular supply from the stalk which ramifies in the endocarp and sends out branches to the inner mesocarp. It is in these conducting channels that one has to look for the deposits.

Experimental

A. Occurrence of Deposits in Fresh Mangoes

Maceration mounts made from 10 varieties of healthy, unhealthy and necrotic mangoes collected from different orchards approximately at the same stage of maturity were carefully examined with reference to occurrence of deposits in ducts, vessels and the neighbouring mesocarp cells. The observations (summarised in Appendix I) showed that variation in the presence, colour, concentration and distribution of deposits correspond to the relative healthiness or necrocity of the examined mango specimens. The occurrence of deposits in ducts and vessels has been described separately since the two types of conducting channels showed differences in their contents and their reaction to the disease.

a. *Necrotic mangoes*

Deposits in xylem. Necrotic mangoes of all stages showed the presence of deposits in the xylem elements. No mango fruit was encountered which, although necrotic, did not show deposits in xylem. The nature (viscosity) of the deposits, their relative abundance and the degree of choking of xylem due to them, however, depended upon the stage of necrosis and the position of xylem in the mango.

In advanced-necrotic mangoes, the principal xylem vessels in the upper-shoulder region were choked with deposits (Fig. 6-9 ; Pl. XI, Fig. 1-2 ; Pl. XII, Fig. 1-2), most of which were viscous to semi-solid, having a colour-range from deep yellow to red (Fig. 10 ; Pl. XII, Fig. 3). Some fluid deposits, light yellow in colour were also observed. Only a few vessels in this region were free from deposits. The immediate branches of the deposit-choked vessels were found to possess less abundant deposits as they descended down the shoulder. In lower-shoulder and upper-trunk regions, the vessels contained fluid, yellow coloured deposits which were relatively less abundant. In lower trunk the deposits showed increase in abundance, viscosity

and intensity of colour, being viscous to semi-solid and deep yellow to red. In the tip region deposits were most copious : vessels were heavily choked with solid, red, brick-red to dark brown, non-granular deposits of a smooth lump-like form (Pl. XII, Fig. 4-6). Deposits were more abundant in the vessels of the outer and the inner mesocarp than the middle mesocarp in all regions of the n rotic mangoes excepting the upper-shoulder (base) and tip region where deposits were equally abundant in all the three zones of the mesocarp.

The pattern of regional distribution of vascular deposits as described for advanced-necrotic mangoes was observed to be repeated in the earlier stages of necrosis ; the abundance, viscosity and colour intensity of deposits decreased with the dimunition in intensity of necrosis (Appendix I). Thus in slightly diseased mangoes the vessels of the upper shoulder contained broken chains of deposits which were mostly yellow coloured and less abundant and less viscous than that in highly necrotic mangoes. The deposits in vessels lower down were even more scanty, fluid and yellow coloured. In the vessels of tip region, even at the first stage of necrosis (aetiolation), deposits were more abundant than in any other region of the mango.

Deposits in ducts : In early stages of necrosis no deposits were found in ducts. This situation was in contrast with that observed in xylem vessels. As the disease advanced, deposits made their appearance in ducts. In its early stage ductular deposits were confined mainly in the tip region of the mango. In more advanced stages, deposits were also found in other regions of the mango. Ducts running along the outer and inner mesocarp (Pl. XI, Fig. 3) contained most of the deposits whereas ducts in the middle mesocarp remained more or less free of them. In the advanced necrotic tips of mango, deposits were uniformly distributed in copious amounts in all ducts irrespective of their position or size, and often the parenchymatous cells lining the ducts were also found full of deposits.

Deposits filled the ducts to a varying degree, leading to their complete or partial blocking. Ductular deposits were solid, occurring as lumps in various stages of aggregation, and at times giving stratified effect. They were usually bright brown in colour, but occasionally shining red deposits were also found. Ductular deposits were not found to occur in the yellow phase.

b. *Unhealthy mangoes*

A large number of unhealthy mangoes belonging to Dasehri, Safeda, Tamburia, Langra, Bombai and Khajli varieties were examined to detect the presence of deposits in ducts and vessels. Deposits occurred in most of the examined specimens ; only a few unhealthy mangoes did not contain any deposits. Deposits occurred,

invariably in xylem vessels, never in ducts. The general pattern of distribution of vascular deposits was the same as in early necrotic mangoes, but the amount of deposits present in unhealthy mangoes was relatively very small.

Vascular deposits were most abundant in the tip region, less abundant in the upper-shoulder and meagre in lower-shoulder and trunk regions. Large number of vessels in the tip region were choked with deposits ; in the upper-shoulder many vessels were choked to small lengths and some were deposit-free, but their secondary branches contained broken deposit-chains. In other regions of the mango the principal vessels contained only scanty deposits. Vascular deposits in the tip region were highly viscous to semi-solid, deep yellow and very rarely reddish ; those in the upper shoulder were slightly viscous and yellow, and those occurring in lower shoulder and trunk regions were fluid and faint yellow.

c. *Healthy mangoes*

Healthy mangoes of Dasehri, Safeda, Tamburia, Khajli, Kishanbhog and Maldaha varieties from Horticulture Garden were carefully examined with reference to the presence of deposits in conducting channels; both vessels and ducts were found to be completely free of any deposits. These observations indicated that the conducting channels of healthy mangoes are not associated with deposits even in its earliest fluid form.

B. Occurrence of Deposits in Mangoes under Preservation

Preliminary examination of necrotic mangoes preserved for over one year in formalin revealed that the preserved specimens contain considerably more deposits than that occurring in fresh mangoes of the corresponding stage of necrosis. Further the healthy mangoes which had not shown any deposits in fresh condition manifested them under preservation. The preservative-induced deposits were found to be different in nature and distribution from those occurring in fresh necrotic mangoes. The action of various preservatives on mangoes with reference to occurrence of deposits was therefore studied in detail and a comparison made with the deposits occurring in fresh mangoes collected from orchards near brick-kilns. The preservatives employed fell under two bases : (i) those that contained formalin as one of the constituents, (ii) the other containing only alcohol. All the 10 varieties of mangoes at different stages of maturity were utilised. The preserved mangoes were examined periodically. The observations (summarised in Appendix II) relate to the deposits formed in Dasehri and Safeda varieties of mangoes.

a. *Healthy mangoes*

Alcohol preserved healthy mangoes failed to reveal any deposits either in the ducts or in the vessels. It was clear that alcohol was not reactive.

Formalin and form-acetic-alcohol preservation, on the other hand, was found to induce formation of deposits in healthy mangoes, which in the fresh condition invariably contained no deposits. The preservative-induced deposits occurred almost exclusively in the ducts. The vessels, except a few at the zone of attachment of stalk, remained deposit-free. The vascular deposits were highly viscous or solid and the colour invariably deep yellow with a tinge of red. Two types of deposits made their appearance in the ducts : (i) those that were mobile, oily and shining red in colour, resolving themselves into globules on application of slight pressure (Fig. 3 ; Pl. XII, Fig. 9), (ii) those that were solid lumps of irregular outline, shining brick-red, displaying in their body a mosaic of shades from dark to very light colour (Fig. 4-5 ; Pl. XII, Fig. 7-8). Deposits of the first type occurred mainly in the outer-mesocarpic ducts ; the second type of deposits occurred in the ducts of outer and inner mesocarp, while ducts of the middle mesocarp remained relatively free. Ductular deposits were more or less equally abundant in all the regions of the mango (the shoulder, trunk and tip) ; in a few cases, however, a higher concentration of deposits occurred in ducts at the zone of attachment of stalk and the tip.

b. *Unhealthy mangoes*

Alcohol-preservation did not show any detectable difference in nature, colour, amount and regional distribution of vascular and ductular deposits from that observed in fresh unhealthy mangoes of corresponding stage of maturity.

Formalin and form-acetic-alcohol preservation showed considerable effect. It brought about an over-all increase in the quantity of deposits as compared to that found in fresh similar mangoes. It also induced intensification of colour and changes in the nature of deposits. Maximum amount of deposits were formed in the ducts, and only to a limited extent in xylem. Mobile, red-coloured, oily deposits, which were of common occurrence in ducts of preserved healthy mangoes, were found to occur in preserved unhealthy mangoes of only immature stage ; with preserved unhealthy mangoes of increasing maturity this type of ductular deposit was found to occur with decreasing frequency. In mature specimens, ductular deposits were of solid type, along with occasional highly viscous deposits. Vascular deposits in the upper shoulder region were mostly brick-red, sometimes yellow, they were more or less deep yellow in the lower shoulder and trunk, and brick-red in the tip region. In general, intensification of colour of vascular deposits from deep yellow to red was found to be correlated with increasing viscosity.

c. Necrotic mangoes

Alcohol preservation of necrotic mangoes of different stages showed no departure from similar fresh specimens either in the nature or the distribution of conducting channel deposits.

Formalin and form-acetic-alcohol preservation of necrotic mangoes of different stages induced a definite increase in the total amount of deposits as compared to that in similar fresh specimens, and also it brought about an intensification of their colour and viscosity. In preserved necrotic mangoes, deposits were copious, occurring either as a homogeneous mass completely choking the lumen of conducting channels for longer or shorter distances or as masses of irregular outline choking the lumen only partly (Pl. XI, Fig. 4-6). Deposits, both in the vessels and ducts, were solid and brick-red ; in vessels some deep yellow deposits also were discernible. Sometimes these occurred as irregular masses showing mosaic of variety of shades in red.

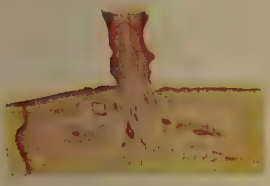
C. Varietal Differences

The foregoing description of the deposits occurring in fresh and preserved healthy, unhealthy and necrotic mangoes, related mainly to the Dasehri and Safeda varieties which were consistently used in all the experiments. An examination of the results obtained with the other varieties showed that there was a general similarity in the occurrence, distribution and nature of the deposits in all the varieties. In these too, under fresh condition, deposits were abundant in necrotic mangoes, less abundant in unhealthy mangoes and completely absent in healthy mangoes. The deposits in different varieties, however, differed in their relative abundance. Arranged in the order of their abundance of deposits in them, the following descending series was obtained : Dasehri, Safeda, Tamburia, Khajli, Langra, Bombai, Kishanbhog, Husnara, Maldaha and Gola. Of these the deposits were most abundant in Dasehri, slightly less in Safeda, followed by Tamburia, Khajli and Langra. In Bombai and other varieties the deposits were very poor, mostly fluid and viscous and exhibited lighter or deeper shades of yellow only. Closely related to the abundance of the deposits was the degree of choking of the conducting system. The vessels of the first two varieties showed maximum choking, and it decreased as the series were descended.

The regional distribution of deposits in ducts and vessels and the nature and colour of deposits of different varieties followed the same trend as in Dasehri and Safeda. Under preservation too all the varieties behaved alike.

D. Deposits in Stalks

The abundance of deposits at the upper shoulder and also at the zone of attachment of the stalk indicated the possibility of the deposits extending into the ducts and vessels of the inflorescence stalk itself. In order to elucidate this point a number of mangoes belonging to different varieties from healthy and diseased orchards were collected with the stalk. Some healthy mangoes were also included. Soon after these had been brought to the laboratory, the stalks were detached



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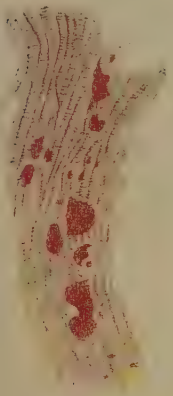

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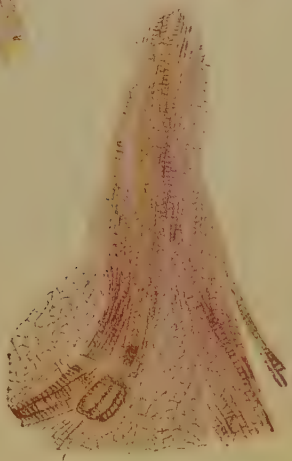

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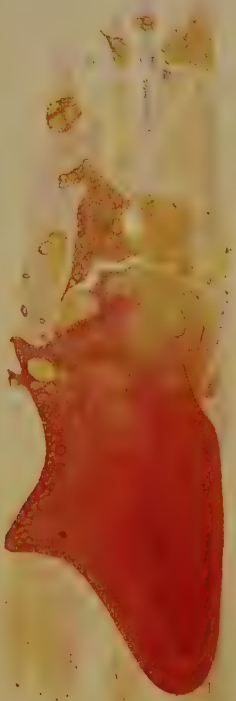

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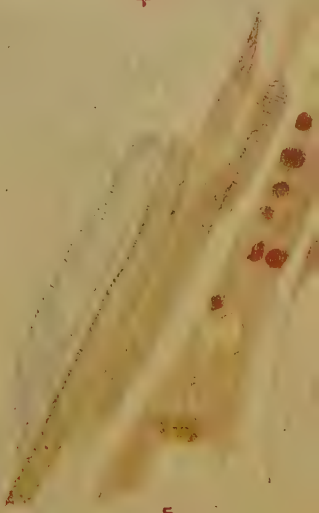

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and each one was macerated separately in fresh condition. A higher concentration of potassium hydroxide was used for the purpose. Examination of macerated stalks revealed that almost all the samples from necrotic and apparently healthy mangoes contained deposits in vessels at the point of attachment of the stalk. The stalks of the healthy mangoes did not show any deposit. The deposits in stalks when present, were of a deep yellow colour ; only rarely had they a reddish tinge. Attempts to trace the deposits further up did not yield conclusive result as out of a large number of stalks examined only two showed coloured fluid in vessels.

E. Chemical Nature of Deposits

Preliminary investigation was made to ascertain the chemical nature of the deposits. The deposits were found not to be reacted upon by concentrated sulphuric, hydrochloric or nitric acids. Alkalies also had no effect on them even on boiling. They were not soluble in any of the organic solvents like acetone, ether and chloroform. The substance appears to be inert.

As a part of the qualitative test, thin sections from various regions were mounted on a slide with a cover-slip and solutions of ferric chloride and osmic acid [Dastur, 1935] were drawn in by means of a filter paper. It was observed that after some time, black colouration developed in certain parts especially in the ducts. The mangoes also showed a positive reaction with copper acetate indicating the probable presence of some kind of tannins, phlobatannins or phlobaphenes, or their derivatives.

Discussion

The regular occurrence of deposits in conducting channels of necrotic mangoes first reported by Das-Gupta and Sinha [1939, 1944], and comprehensively studied during the present investigation, is an interesting feature of mango necrosis. The formation of accumulates or deposits in tissues of plants affected by pathogens [Cook, 1928 ; Wilson, 1933 ; Miller, 1938 ; York, 1938 ; Edgerton Carvajal, 1944], or due to excessive salt accumulation [Broek 1937], or due to physical injury is well-known. Thrupp [1928] reports the occurrence of a plugging substance in the vessels of hops affected with mosaic disease. Esau [1933], in her study of curly top of sugar beet (*Beta vulgaris*), refers to a material which originates in the necrotic phloem and escapes into the xylem through intercellular spaces. Chatterjee [1943] records the occurrence of an yellowish brown amorphous material in necrotic areas of papaya (*Carica papaya*). Eltinge [1941] reports the plugging of xylem cells in stem and veins of leaves of tomato (*Lycopersicum esculentum*) plants affected with manganese deficiency. Dastur [1939] found accumulation of tannins in cells of leaves and roots of diseased cotton plants that produced prematurely cracked bolls.

While deposits never occur in fresh healthy mangoes, they can always be detected in mangoes showing the earliest stages of necrosis. Even among unhealthy mangoes (apparently healthy mangoes growing in diseased orchards), similar type of deposit (though relatively less in amount) could be detected in

some specimens. The presence of deposits in these unhealthy mangoes confirms the earlier suggestion by Das-Gupta and Sinha [1944] that the deposits are the first internal index of the disease.

In fresh mangoes showing the earliest stages of necrosis, deposits are found in xylem but not in ducts, while in later stages of necrosis, deposits occur both in xylem and ducts, largely in their principal tributaries supplying the outer and inner mesocarp; in the middle mesocarp where the conducting channels are fewer, deposits too are considerably less abundant. In individual necrotic mangoes the maximum amount of deposits are found in the tip region, less in the upper shoulder and relatively poor in the lower shoulder and trunk regions. It is interesting that notwithstanding abundant deposits in the upper shoulder, the advancing necrotic area starting from the tip stops short of this region.

Increase in abundance of deposits in xylem of fresh necrotic mangoes is found to correspond with a parallel aggravation of necrosis, and linked with these are *pari passu* changes in colour and concentration (viscosity) of deposits. Xylem deposits when poor in amount (in the earlier stages of necrosis), are fluid and light yellow; with increasing concentration (parallel with intensification of necrosis), they become progressively viscous and deeper yellow, and passing from viscous to semi-solid states, the colour ranges from reddish to brick-red. In very advanced stages of necrosis the solid xylem deposits become dark brown. Ductular deposits in fresh mangoes occur only in advanced stages of necrosis; they are solid, bright brown, occasionally reddish but never yellow.

In the stalk of necrotic mangoes, pale yellow and fluid deposits occur upto a certain distance, but it is not clear if the presence of deposits in stalks is due to backward translocation of deposits, deposit-precursors or deposit-inducing agents from the mango fruit or whether they originate initially in the stalk itself.

The effect of preservatives on the formation of deposits in mangoes is interesting. Dastur [1939] observed the appearance of precipitates in formalin-preserved leaves and roots of cotton plants. Similar preservative-reaction was observed in mango fruits when preserved in formalin-containing preservatives. Formalin and form-acetic-alcohol preservation was found to induce deposit-formation in conducting channels of healthy mangoes which never contained any deposits in the fresh condition; in preserved necrotic mangoes a definite increase in amount of conducting channel deposits over that occurring in the fresh condition was observed. It was, however, observed that formation of deposits induced by preservatives occurred predominantly in the ducts, and very little in the xylem. In fresh necrotic mangoes, on the other hand, deposits are found mainly in the xylem; and occurrence of deposits in ducts and in the parenchymatous cells of the necrotic region in advanced stages of necrosis is likely to be due to injection from the xylem vessels. This points to a fundamental difference between the formation of deposits in necrotic mangoes in the fresh condition and in mangoes under preservation. The implication of formation of deposits in ducts and to a less extent in vessels under formalin-preservation is not clear; it seems that ' Chep ', which is the main content of the ducts is more reactive to the action of formalin than the contents of xylem vessels.

The deposits in both vessels and ducts of formalin-preserved mangoes, while resembling in general those that occur in fresh necrotic mangoes, are, however, usually solid and irregular in outline. The preservative-induced deposits in xylem range from viscous and yellow to solid and brick-red. Preservative-induced deposits in ducts are of two types, firstly solid and brick-red, and secondly mobile, oily and shining red. The 'oily' ductular deposits do not occur in fresh mangoes at any stage of necrosis, and in preserved mangoes are found only in healthy and immature unhealthy fruits.

All varieties of mangoes examined have shown the same type of conducting channel deposits, but frequency and amount of the deposits contained in them varied. Arranged in order of diminishing abundance of conducting channel deposits, the following series was obtained: Dasehri, Safeda, Tamburia, Khajli, Langra, Bombai, Kishanbhog, Husnara, Maldaha and Gola.

The preliminary chemical investigation carried out has shown that the deposits belong to a group of tannins, phlobatannins, phlobaphenes or their derivatives. The accumulation of tannins in plant tissues is not uncommon and has been reported in the leaves of American cotton [Dastur, 1939], celery bulbs [Butler, 1918], *Pinus sylvestris* [Hutchinson, 1935].

The present investigation has shown that the formation of deposits in conducting channels of mango is constantly associated with the incidence of necrosis from the earliest stages, or even before the necrosis is externally expressed (in some unhealthy mangoes), and increase in their amount corresponds significantly with the aggravation of necrosis. The fact that unhealthy mangoes which contain internal deposits do not show any external symptoms of necrosis can perhaps be attributed to the lack of critical deposit concentration required for the production of external disease symptoms; differential disease resistance of individual fruits may be another factor. The complete absence of deposits in some unhealthy mangoes indicates that these mangoes have escaped or resisted necrosis, and are perfectly healthy.

The precise relation of conducting channel deposits to mango necrosis is still obscure. On one hand, it is possible that the deposits are secondary effects of necrosis, at least in the final stages; on the other, necrosis may be the result of choking of conducting channels by deposits. Apparently the choking of conducting channels by deposits disrupts the translocation mechanism of the mango and the ensuing metabolic disturbance may well be the immediate cause of necrosis of tissues at the tip end of the fruit. This metabolic disturbance is also reflected in the growth of affected mango fruits which fail to attain the normal size.

The exact cause and mode of deposit-formation in conducting channels of necrotic mangoes is unelucidated. A constituent of the brick-kiln fumes, hitherto unidentified chemically, has been suggested by Das-Gupta *et al.*, [1950] as the possible toxic chemical that may react with the contents of conducting channels to form deposits and ultimately cause necrosis of mangoes.

Summary

The paper presents in detail the results of a comprehensive histopathological investigation of 10 different varieties of necrotic, unhealthy and healthy mangoes in fresh and preserved conditions, with reference to presence and distribution of deposits. All varieties gave more or less similar results.

Deposits are invariably present in necrotic mangoes. In earlier stages of necrosis deposits occur only in vessels; in later stages they occur both in vessels and ducts, and in very advanced stages also in the parenchyma. Concentration of deposits increases with the advance of necrosis.

Some unhealthy mangoes (externally non-necrotic, but collected from diseased orchards) contain no deposits; others contain in their vessels but not in ducts variable amounts of deposits of the same type as in mangoes showing first symptoms of necrosis.

Deposits are not found in healthy mangoes.

Formalin-preservatives are reactive. They induce formation of deposit in healthy mangoes, which otherwise do not contain deposits, and in unhealthy and necrotic mangoes, which already contain deposits. Preservative-induced deposits occur mainly in ducts whereas deposits in fresh condition are primarily found in vessels. Alcohol is a non-reactive preservative.

Deposits commonly occur in outer and inner mesocarp which is richly supplied with conducting channels: deposits are infrequent in the middle mesocarp which has relatively poor vascular supply. Maximum deposit-concentration occurs in the tip region of mango where the symptoms of necrosis first appear. Deposits are less copious in the upper shoulder (base) and relatively poor in the lower shoulder and trunk regions. Deposits are also found in the vessels of stalk of necrotic mango.

In fresh mangoes, vascular deposits in low concentration are fluid-light yellow; with increasing concentration they pass from viscous-deep yellow to semi-solid and solid, brick-red types. In final stages of necrosis, solid vascular deposits become dark brown. Ductular deposits in fresh mangoes are stratified-solid, bright brown, occasionally reddish, but never yellow. In formalin-preserved mangoes, besides normally-occurring afore-mentioned types of deposits, preservative-induced deposits are found which are (i) oily, shining red (in ducts only) and (ii) solid lump-like in various stages of aggregation (both in vessels and ducts).

Preliminary chemical investigation shows that the deposits belong to a group of tannins, phlobatannins or phlobaphenes or their derivatives.

The available data suggest that the deposits are casually related to necrosis and that their presence in fresh mangoes is the first internal index of the disease before it is externally expressed.

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APPENDIX I

Distribution of deposits in vessels and ducts of mango fruits as observed under fresh condition

Regions of fruit	Condition of fruits															
	Necrotic—Advanced						Necrotic—Early				Unhealthy				Healthy	
	Nature of deposits (if any) in :						Nature of deposits (if any) in :				Nature of deposits (if any) in :				Nature of deposits (if any) in :	
	Vessels			Ducts			Vessels		Ducts		Vessels		Ducts		Vessels	Ducts
1. Upper shoulder (or 'base')	Copious in continuous chains	Viscous to semi-solid	Deep yellow to reddish	Poor to abundant	Solid (stratified)	Bright brown. Rarely shining red, never yellow	Copious in broken chains	Viscous to semi-solid	Deep yellow	Absent	Poor	Viscous	Deep yellow	Absent	Absent	Absent
2. Lower shoulder	Poor	Fluid	Yellow	Poor	Solid	Bright brown	Poor	Fluid	Yellow	Absent	Poor	Fluid	Light yellow	Absent	Absent	Absent
3. Upper trunk	Poor	Fluid	Light yellow	Poor	Solid	Bright brown	Poor	Fluid	Light yellow	Absent	Poor	Fluid	Light yellow	Absent	Absent	Absent
4. Lower trunk	Abundant	Viscous to semi-solid	Deep yellow to red	Poor to abundant	Solid	Bright brown	Abundant	Viscous	Yellow	Absent	Less poor	Viscous	Deep yellow	Absent	Absent	Absent
5. Tip	Copious	Solid	Reddish to brick-red, finally dark brown	Abundant to copious	Solid (stratified)	Bright brown to shining red, never yellow.	Copious	Semi-solid	Reddish to brick-red	Absent	Abundant	Highly viscous to semi-solid	Deep yellow, very rarely reddish	Absent	Absent	Absent

APPENDIX II

Comparative distribution of deposits in vessels and ducts as observed under fresh and preserved conditions

Conditions of observation	Changes in presence and nature of deposits in mangoes							
	Healthy		Unhealthy		Necrotic—Early		Necrotic—Advanced	
	Vessels	Ducts	Vessels	Ducts	Vessels	Ducts	Vessels	Ducts
1. Fresh	Absent	Absent	Present—fluid to solid, light yellow to deep yellow	Absent	Present—viscous to semi-solid, yellow to reddish	Absent	Present—viscous to solid yellow to brick-red, finally dark brown	Present—solid (Stratified), bright brown to shining red
2. Preserved in 90 per cent alcohol	Absent	Absent	Present—same as in fresh	Absent	Present—same as in fresh	Absent	Present—same as in fresh	Present—same as in fresh
3. Preserved in 4 per cent formalin and form-acetic-alcohol	Mostly absent—Where present (at the zone of attachment of stalk) are highly viscous at times solid. Colour invariably deep yellow with tings of red	Present—deposits of two kinds: (i) Mobile, oily, shining red, (ii) Solid, shinning brick-red	Present in increased amounts—highly viscous to usually solid, deep yellow to red	Present—mostly solid and deep yellow to red. Oily mobile red-coloured deposits found only in immature fruits	Present in increased amounts—solid, brick-red, some deep yellow	Present—solid brick-red	Present in increased amounts—solid, brick-red, some deep yellow	Present in increased amounts—solid, brick-red

AN EXPERIMENTAL INVESTIGATION ON SAMPLING FOR YIELD, TILLERS AND HEIGHT IN REPLICATED FIELD EXPERIMENTS ON RICE

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(With two text-figures)

ESTIMATES of plant attributes in replicated field experiments are often required to find out the effect of various treatments on these characters. So far as rice crop is concerned, tillers and height are two characters which are frequently estimated by sampling. Although, yield is generally obtained by complete harvest, which is often more convenient than sampling, sometimes sampling is also used for estimation of plot yields. The determination of suitable sampling unit, method of sampling and amount of sampling are important aspects to be decided in a sampling programme. Apart from a few investigations by the Agricultural Meteorology Section at Poona [Kalamkar *et al.*, 1943 ; Sreenivasan, 1950], no other work on sampling studies with rice crop in field experiments is reported so far. Kalamkar *et al.* found that a positive intraclass correlation existed between adjacent bunches, and so they suggested for future use a unit of type,

O *xxx* O *xxx* O *xx*
omit omit omit
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This modified unit was tried along with seven other types by Sreenivasan [*loc. cit.*] for estimation of yield who found that the suggested structure was the best among those tried. This unit was, therefore, recommended for sampling observations in coordinated 'Crop Weather Scheme' experiments. However, this unit being somewhat complicated, is not very convenient for general adoption. Square or rectangular sampling units, which are much more convenient in practice, are very often adopted for sampling in field experiments. The object of this paper is to present the results of sampling investigation conducted at the Central Rice Research Institute, Cuttack, to compare square units of different sizes, along with some other structures including the one recommended for 'Crop Weather Scheme'.

Material and Method

For the purpose of the present investigation, a field of size 128 ft.×66 ft. at the Institute farm was uniformly planted with 6 ft.×6 ft. spacing, using one plant per hole. The variety used was T. 1145, a medium duration local variety. The gap filling, weeding, etc., were done as usual and the crop growth was quite satisfactory. For experimental purpose, a net area of 120 ft.×60 ft. was marked out, leaving the rest as border. Twenty-five plots of 24 ft.×12 ft. were obtained by dividing the field suitably.

Each plot was further sub-divided into two half-plots of 12 ft. × 12 ft. In each half-plot, two square samples of 3 ft. × 3 ft., two sampling units of the type recommended by the Agricultural Meteorology Section, and 10 individual plants were marked at random. The following observations were taken individually for each plant in the samples and recorded along with the position of the plant. The observations taken were : (*a*) tillers and height about six weeks after planting, and (*b*) number of panicles and yield at harvest. The observations at the latter stage were also taken in each half-plot on 18 plants at regular intervals along the rows, the first plant being taken at random. From each 3 ft. × 3 ft. sampling unit, smaller square sampling units were formed by deleting successively the last row and column. Sampling units of different sizes and structures which were studied are given in Table I :

TABLE I

Number and structure of each sampling unit

Serial No.	Structure of sampling unit
I	3 ft. × 3 ft.
II	2½ ft. × 2½ ft.
III	2 ft. × 2 ft.
IV	1½ ft. × 1½ ft.
V	1 ft. × 1 ft.
VI	Six contiguous plants in line as 0 0 0 0 0 0
VII	0 X X X 0 X X X 0 X X X X 0 X X X 0 X X X 0
VIII	Four corner plants of a 2 ft. × 2 ft.
IX	Random plants
X	Systematically taken plants

The symbol 'X' in No. VII denotes a plant on which no observation was taken

The underlying theory of sampling in replicated field experiments is given by Yates and Zacopany [1935]. If Y_{ijk} is the yield of the k^{th} sample in the j^{th} treatment plot of i^{th} block, then the mathematical specification is $Y_{ijk}=r+b_i+t_j+P_{ij}+s_{ijk}$ ..... (1) where b_i and t_j are constants for the block and treatment effects, p_{ij} is the component of experimental error which is common to all samples of the $(i, j)^{th}$ plot and s_{ijk} is the additional component of error which varies from sample to sample for a given plot independently of p_{ij}. We can assume $E(p_{ij})=E(s_{ijk})=0$

where E denotes expectation. It is not possible to estimate the quantities 'p_{ij}', and 's_{ijk}', but the analysis of variance provides estimates of variances of 'p_{ij}' and 's_{ijk}'. Denoting these variances by V_p and V_s respectively, the variance of the mean (y_n) of n samples per plot is given by

$$V(y_n)=V_p+\frac{V_s}{n} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

If there are N possible sampling units in a plot, the variance of the mean of all the N samples per plot is given by $V(y_n)=V_p+\frac{V_s}{n}$ ..... (3)

The relative amount of information obtained with sampling is,

$$\frac{V_p+\frac{V_s}{N}}{V_p+\frac{V_s}{n}}=\frac{1}{1+c\frac{(1-f)}{f}} \quad \ldots 4$$

where 'f' is the sampling fraction, $\frac{n}{N}$, and c is the fraction of the total error variance due to variation between sampling units when the whole plot is harvested,

$$\text{i.e. } \frac{\frac{V_s}{N}}{V_p+\frac{V_s}{N}}$$

In the present study, the 25 plots were grouped into five blocks of five contiguous plots each. The analysis of variance was carried out with each set of data to estimate the components of error variance and these estimates were utilized to find out the optimum sampling unit and the amount of sampling necessary to estimate the treatment means with a given level of accuracy. The form of the analysis of variance is shown below:

Analysis of variance table

Source of variation	D.F.	M.S.	Expected value of M.S.
Blocks	4	B	
Plots within blocks (experimental error)	20	E	$4V_p+V_s$
Sub-divisions within plots	25	D	$2V_d+V_s$
Samples within sub-divisions	50	S	V_s
TOTAL	99		

V_d is the true variance between sub-divisions. Estimates of V_p and V_s are $\frac{E-S}{4}$ and S, respectively.

In the case of units IX and X each individual plant has been taken as the unit and the degrees of freedom in the analysis of variance for samples within sub-divisions has been modified accordingly. In the case of unit X, although the individual plants cannot be considered at random, the variation between plants has been found to compare with the variation between random plants obtained from structure IX.

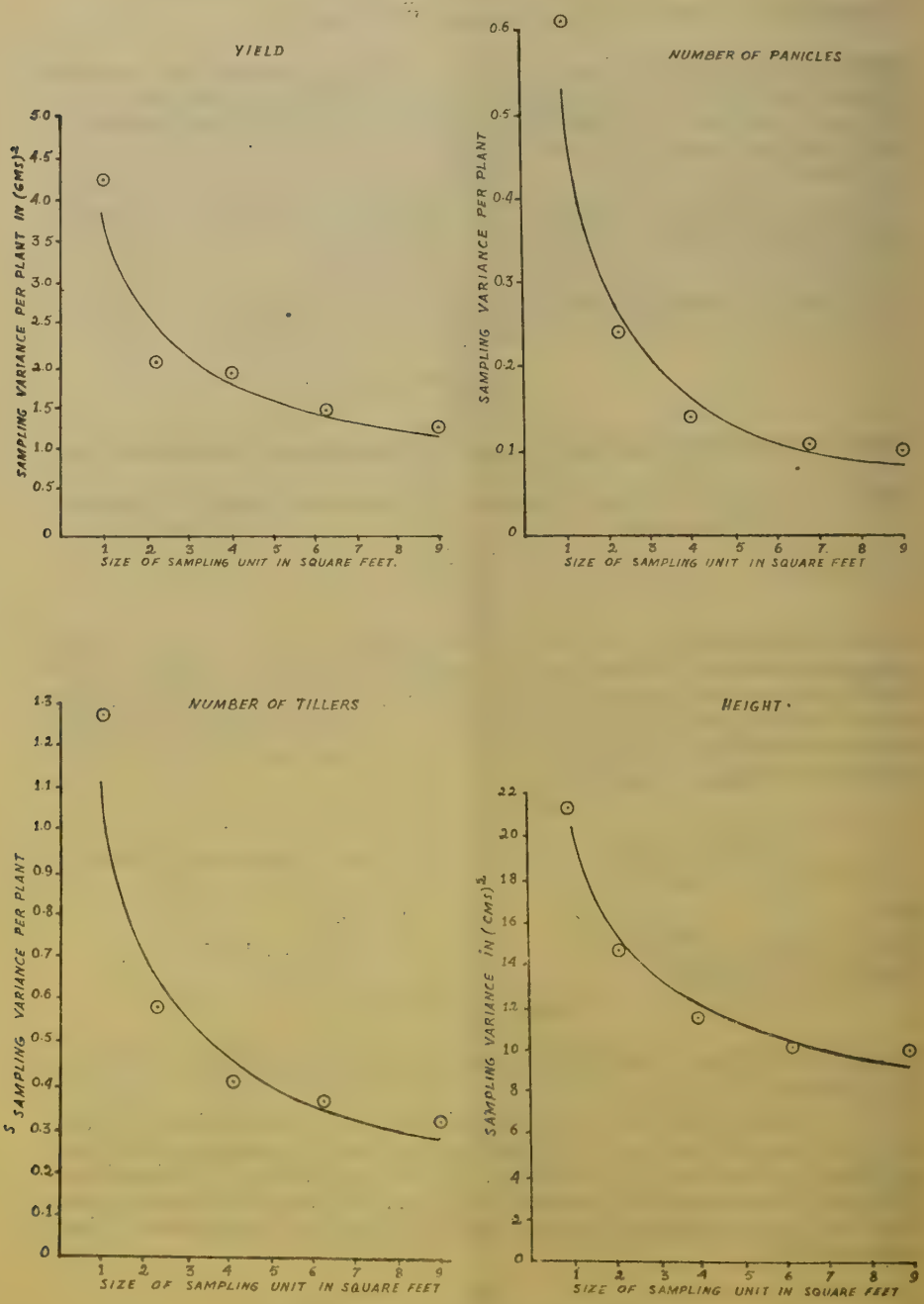

FIG. 1. Fitted curves and the observed values

Results

Table II gives the estimates of mean H_y, sampling variance V_s, and true plo t variance V_p.

Table II

Sampling structure and statistical data relating to plant characters

Sampling structure	Plant characters											
	Yield in grams per plant			Number of panicles per plant			Number of tillers per plant			Height in cm.		
	y	V_s	V_p	y	V_s	V_p	y	V_s	V_p	y	V_s	V_p
I	7·23	1·26	0·19	3·79	0·10	0·023	6·47	0·32	0·030	70·87	9·92	7·31
II	7·30	1·46	0·21	3·81	0·11	0·040	6·53	0·37	0·065	71·19	10·18	5·16
III	7·27	1·91	0·01	3·78	0·14	0·014	6·46	0·40	0·094	69·01	11·56	5·90
IV	7·14	2·06	0·37	3·73	0·24	0·055	6·42	0·58	0·040	70·78	14·77	6·07
V	6·94	4·23	0·62	3·67	0·61	0·073	6·62	1·27	0·590	70·80	21·37	6·32
VI	7·06	4·49	0·40	3·69	0·46	0·050	6·46	0·98	0·213	70·92	12·14	7·99
VII	7·27	3·25	..	3·83	0·41	0·008	6·47	0·64	0·183	70·86	8·88	6·95
VIII	7·28	5·75	..	3·78	0·38	0·108	6·54	1·24	0·138	70·94	14·36	4·41
IX	7·29	15·30	1·02	3·79	2·39	0·008	6·51	6·35	0·327	70·59	45·08	11·18
X	7·15	14·59	0·59	3·80	2·37	0·035	..	..	..	..	..	..

(i) *Size of sampling unit and sampling variance*

The sampling units from I to V in Table II are square units of different sizes. We find that for all characters, the sampling variance decreases with increased size of unit. It is also seen from Table II that the mean values and plant to plant variation within plots for random plants and systematically taken plants are nearly the same. Equations of the form $Y=ax^{-g}$, where 'x' is the size of the sampling unit and 'y' is the corresponding sampling variance, were fitted to the data, by linear regression technique after transforming both variables to logarithmic values. The constant 'a' is the value of the sampling variance when the sampling units are of unit size, and 'g' is a measure of the association between contiguous units forming a larger sampling unit. When g=1, contiguous units behave as though they are independent, while a value of 'g' less than unity indicates positive correlation between contiguous units—greater being the correlation, smaller the value of 'g'. Hence when 'g' is small large sampling units will be inefficient. The regression analysis is given in Table III. The fitted curves and the observed values are shown in Figure 1. It is clear that the fit is quite satisfactory in all cases.

The values of 'g' obtained are given below for each character :

Character	'g'
Yield	0·5237
Number of earheads per plant	0·8402
Number of tillers per plant	0·6202
Mean height	0·3229

TABLE III

Analysis of variance for regression

Source of variation	D.F.	Yield			Earhead			Tiller			Height		
		S.S.	M.S.	F	S.S.	M.S.	F	S.S.	M.S.	F	S.S.	M.S.	F
Due to regression	1	0·1557	0·1557	48·66	0·4008	0·4008	69·1	0·2184	0·2184	43·68	0·0747	0·0747	83·00
Deviations from regression	3	0·0096	0·0032	..	0·107	0·0058	..	0·0150	0·0050	..	0·0027	0·0009	..
TOTAL	4	0·1653	..	..	0·5082	..	..	0·2334	..	..	0·0774	..	..

F. 05 for n1=1; n2=3 is 10·13

From the magnitude of the 'g' values given above, it is clear that correlation between contiguous units is maximum for height followed by yield and, least for number of panicles.

(ii) *Choice of sampling unit*

With a given sampling fraction, the minimum variance is generally obtained with smaller units. If Ve is the variance between individual plants in the plot, the variance between sampling units consisting of n plants will be equal, greater or less than $\frac{Ve}{N}$ according as the correlation between plants within the unit is zero, positive or negative. Table IV gives the observed sampling variances of the units consisting of four plants with the corresponding variances calculated on the basis of no correlation between the plants.

TABLE IV

Sampling variances of the units

Variance	Yield	No. of ear-heads	No. of tillers	Height
Sampling variance with units of four adjacent plants when there is no correlation between the plants	3·82	0·60	1·59	11·27
Observed sampling variance	4·23	0·61	1·27	21·37

It is clear from Table IV that for height, samples of four adjacent plants will be very inefficient, but not so for the other characters specially, for tillers and number of panicles. Taking into consideration the amount of labour required for trampling of the plot if a large number of individual plants are taken, we may take for characters other than height, sampling units consisting of suitable number of adjacent plants, without any appreciable loss of efficiency. It can also be seen from Table II, that for these three characters the sampling variances obtained with the structure recommended for the 'Crop Weather Scheme' (Structure VII), is not appreciably different from the more convenient square units IV and V, when adjustments for the number of plants in the units are made. The square units have further advantage that they directly give estimates per unit area also.

The best size of sampling unit is that which minimises the experimental error for a given cost. It is generally not possible to give exact figures for the cost involved at different stages in sampling. The cost of sampling can be classified as : (*a*) cost of locating the sample and moving from sample to sample, and (*b*) cost of taking the observations, recording and computing. Item (*a*) is fixed whatsoever be the size of the unit and item (*b*) can be assumed to increase approximately in

proportion to the size of the sampling unit. Denoting costs under (*a*) and (*b*) by C_o and C_1, the total cost C per plot, of taking n_x samples each of size 'x' units per plot is given by :

$$C=n_x(C_1x+C_o)$$

The experimental variance of the individual plot mean of n_x samples per plot is given by :

$$V_x=V_p+\frac{V_s}{n_x} \quad \ldots\ldots(1)$$

The optimum value of 'x' will be that value which minimises (1) subject to the conditions :

$$n_x(C_1x+C_o)=C \quad \ldots\ldots(2)$$

$$Vp+\frac{Vs}{N/x}=K \quad \ldots\ldots(3)$$

where 'N' denotes the total number of samples of unit size in each plot, and hence N/x the total number of samples of size 'x' units, 'C', being the assigned cost per plot and K, being the variance on unit basis with complete count of the plot. The two quantities are fixed quantities. After replacing V_s, by ax^{-g} the required value of 'x' will be found to be :

$$x_o=\frac{(C_o/C)g}{(C_1/C-1/N)(1-g)} \quad \ldots\ldots(4)$$

If the sampling fraction is very small, as is usually the case, we may neglect 1/N compared with C_1/C and C_o/C in which case

$$x_o=\frac{C_o}{C_1}\quad\frac{(g)}{(1-g)} \quad \ldots\ldots(5)$$

This depends only on the ratio of the overhead cost to the varying cost and the value of 'g'.

In the case of tillers and number of panicles, each plant is counted separately and recorded, the observations being over in the field itself, while in the case of yield, the whole unit is harvested and the number of plants counted. The operations of threshing, drying and storing separately sample yields and their weighments have to be done. With the increased size of sampling unit the additional cost involved in harvesting and threshing is very small, while the labour involved in drying, storing and weighing is not increased. Taking all these factors into consideration, we may roughly give the value of C_o/C_1, as given in Table V, although these might vary with several factors such as field and crop condition, and the available facilities for such operations as harvesting, threshing, drying, weighing, etc.

TABLE V

Optimum size of sampling units

Character	$\frac{\text{Overhead cost}}{\text{Varying cost with increased size}} = (C_0/C_1)$	Optimum size of sampling unit in sq. ft. $x_0 = (Co/C_1)\frac{g}{1-g}$
Yield	16·00	16·1
Number of panicles	1·50	7·9
Number of tillers	1·33	2·3

If the correct formulae for the optimal size were used, the values would have been slightly higher than those given in the table. We may take for practical purposes, 4 ft.×4 ft., 3 ft.×3 ft. and 2 ft.×2 ft. units for yield, number of earheads and number of tillers respectively. Since the largest sampling unit tried was 3 ft.× 3 ft., the optimum value given above for yield is an extrapolated value. However the extrapolation could be taken as satisfactory in view of the close agreement of the observed variances with those obtained by the formula ax^{-g}. Height of the crop is usually taken along with the tiller count. We have already seen that the heights of the neighbouring plants are highly correlated. In view of this correlation and the general lower variability of height, it is sufficient to take for height measurement, a sub-sample from the 2 ft.×2 ft. unit used for tiller count. To take a random sub-sample will be inconvenient and we may, therefore, take the four plants nearest to the four corners of the primary unit. From Table VI which gives the means and variances obtained with four random plants and four corner plants of a 2 ft.×2 ft. unit it is evident that there is no appreciable difference between the two.

TABLE VI

Sub-sampling for height from a 2 ft.×2 ft. primary sampling unit

	Four random plants	Four corner plants
Mean height of the plant in cm. (x)	11·35	70·94
Sampling variance (V_s)	12·57	14·56
True plot variance (V_p)	4·65	4·41

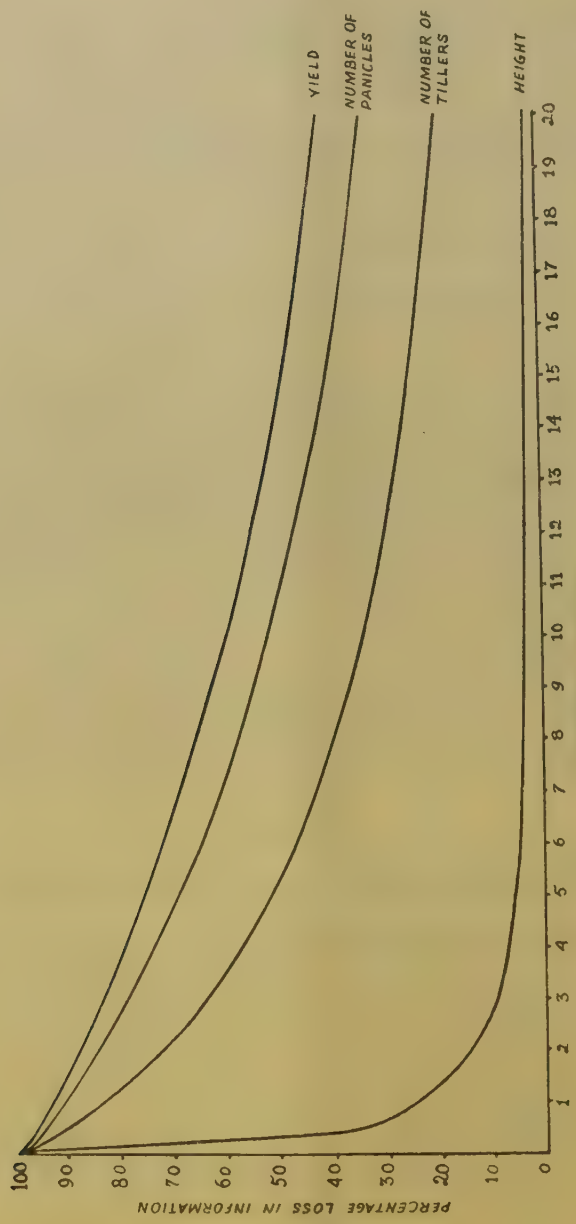

Fig. 2.—Percentage loss in amount of information obtained with different percentage sampling of characters

(iii) *Amount of sampling and information obtained*

The relative amount of information (I) obtained by taking a sampling fraction is, $I = \frac{1}{1+c\frac{(1-f)}{f}}$ where 'c' is the fraction of the total error variance between sampling units when the whole plot is harvested. Figure 2 shows the percentage loss in amount of information obtained with different percentage sampling for all the characters. The sampling structures used were 3 ft.×3 ft. for yield and number of panicles, 2 ft.×2 ft. for number of tillers and four corner plants of a 2 ft.×2 ft. square for height. It will be seen that for height, the loss in information is very small even with only 1 per cent sampling.

Number of samples required for varying levels of accuracy

From formula (2) it can be seen that the minimum number of samples 'n' required to estimate the mean value with a S.E. of 'p' per cent is given by :

$$n = \frac{(\text{percentage of sampling error})^2}{rp^2 - (\text{percentage true plot error})^2}$$

where 'r' is the number of replications.

Table VII gives the minimum number of samples necessary for estimating the mean values with different levels of accuracy. In agronomic experiments, the number of replications is usually fixed in advance, and ranges ordinarily from four to eight depending on other considerations.

TABLE VII

Minimum number of samples for estimating the mean values

No. of replication	Yield sampling unit : 3 ft.×3 ft.					Number of panicles 3 ft.×3 ft.			Tiller 2 ft.×2 ft.			Height 4 corner plants of a 2 ft. × 2 ft. square			
	SE per cent					SE per cent			SE per cent			SE per cent			
	4	5	6	7	8	3	4	5	3	4	5	2	3	4	5
4	11	5	3	2	2	4	2	1	8	3	2	4	2	1	1
6	5	3	2	2	1	2	1	1	4	2	1	2	1	1	1
8	4	2	2	1	1	2	1	1	2	1	1	2	1	1	1

In Table VII it is indicated that with usual number of replications and about four samples per plot, the mean values for all characters are estimated with S.E. not exceeding 5 per cent. In the case of height which is the least variable character, S.E. of the mean is even less than 2 per cent. Thus we find that with the amount of sampling and types of sampling unit indicated above, treatment differences of the order of 12 to 15 per cent for tiller, number of panicles and yield, and 4 to 6 per cent for height can be detected.

Summary

The results obtained in an experiment with rice on sampling for yield, tillers and height, conducted at the Central Rice Research Institute, are given.

The logarithm of the sampling variance was found to decrease linearly with increase in size of sampling unit from 1 ft. × 1 ft. to 3 ft. × 3 ft. for all characters, the rate of decrease being least for height and highest for number of panicles.

The sampling structure recommended by the Agricultural Meteorology Section in connection with Crop Weather Scheme was not found superior to other more convenient units.

Random plants and plants systematically taken along the rows gave almost the same mean and variance indicating thereby lack of any systematic trend along the rows.

Taking into consideration the costs incurred at different stages of sampling, the optimal size of sampling unit was found as 4 ft. × 4 ft., 3 ft. × 3 ft., 2 ft. × 2 ft. and four corner plants of a 2 ft. × 2 ft. square respectively, for yield, number of panicles, tillers and height.

With about four samples per plot and four to six replications available, treatment differences of the order of 12 to 15 per cent for yield, tillers and number of panicles, and about 4 to 6 per cent for height could be detected.

Finally it may be mentioned that estimates of yield and tillers are more often required on unit area basis rather than per plant basis. The date obtained here has been accordingly utilized to study the sampling variances, etc., on this basis. Although there were slight differences due to variations in stand, the general conclusions given here have been found to hold good in this case also. Therefore, the results regarding optimum size of sampling unit, number of samples, etc., could be used for this purpose also.

Acknowledgement

We are very grateful to Dr N. Parthasarathy, Director, and Dr Moti V. Vachhani, Agronomist, Central Rice Research Institute, Cuttack, for having very kindly provided us with facilities to conduct this investigation and giving helpful suggestions. We are also thankful to Dr V. G. Panse, Statistical Adviser, Indian Council of Agricultural Research, New Delhi, for going through the manuscript and giving useful suggestions.

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A COMPARATIVE STUDY OF THE EFFECTS OF TYPES OF 'SEED' ON RATE OF EMERGENCE, ESTABLISHMENT OF PLANTS AND YIELD IN DIFFERENT *DASHEENS* GROWN IN EGYPT

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TWO types of *dasheen* are grown in Egypt, viz., the Egyptian (*Colocasia antiquorum*) and the American (*Colocasia esculenta*). The former is one of the established food plants of Egypt since the beginning of Christian era. Its leaves are erect, broadly ovate and light green in colour. In the American *dasheen* leaf, there is a purplish spot at the point of attachment of the petiole with the blade. The Egyptian *dasheen* is characterized by a great amount of mucilaginous substance as compared to the American.

The literature shows that little work has been done on *dasheen*. Mahmoud [1953] found that the cormel and the upper part of the corm produced a significantly higher yield of *dasheen* than corm piece.

The investigations reported in this paper were undertaken to determine the rate of emergence, the establishment and the yield in the two types of *dasheen* using three types of corm pieces namely, the cormel, apical piece and corm piece.

Materials and Methods

The total number of treatments were six as there were two types of *dasheen* and three types of "Seed".

Each seed weighed four ounces. Six factorial treatments were arranged in a randomized block design in four replications. Berseem was ploughed in the soil and 200 kg. of superphosphate per**feddan* was applied to seed bed. Sodium nitrate was given to the crop at the rate of 200 kg. per *feddan* after three months of planting. Stable manure at the rate of three tons per *feddan* was dressed to the crop at the end of July when splitting of ridges was being done. Plants were irrigated whenever they required water. Planting was carried out on March 19, 1953, at Alexandria University Agricultural Experimental Farm. Each plot consisted of 4 ridges, 88 cm. apart and 1000 cm. long. The total number of plants of the two centre rows were counted at two days interval after emergence. The germination rate index of every plot was calculated according to Bartlett [1937] and these indices were subjected to the analysis of variance. Number of cormels, as well as the total yield was recorded at harvest on December 10, 1953.

**Feddan* is a unit of land measurement in Egypt and equals 6144·5 square yards.

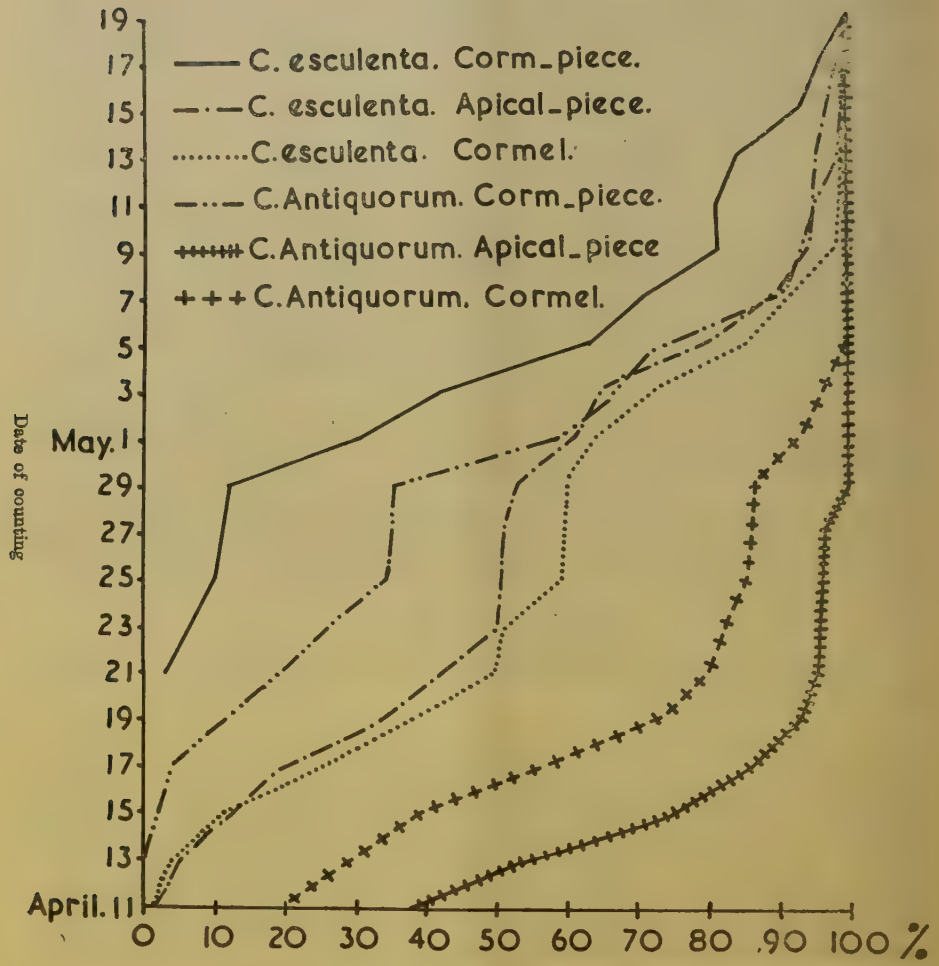

Fig. 1 Percentage of stand of plants by stated dates.

Results and Discussions

Rate of plant emergence

Table I

*Germination rate index**

Treatment	Germination rate index
Egyptian *dasheen*	0·784
American *dasheen*	0·598
C. D. at 5 per cent level	0·036
Apical piece	0·788
Cormel	0·757
Seed piece	0·527
C. D. at 5 per cent level	0·045

Table I shows that there was significant difference in germination rate index due to types of corm pieces and types of plants. Egyptian *dasheen* emerged earlier than the American while cormels and apical pieces came up before corm pieces.

It is clear from Fig. 1 that plant emergence covered about 40 days after planting. The difference in rate of emergence may be due to many factors such as starch sugar ratio, hormones present, dormancy of buds, amount of stored food, bud position on the corm and probably other factors which need further study.

The rates of germination, i.e., the number of days, within which 50 per cent of germination took place, was in the case of American *dasheen*, 45, 35 and 39 days for corm piece, cormels and apical pieces respectively, while in the Egyptian *dasheen*, these were 42, 28 and 24 days respectively.

The germination curve (Fig. 1) shows that the germination power expressed as per cent of seed germinating within a given period of corm piece was less than that of apical piece. The difference in the condition of reserved food and probably the enzymes present in the apical piece compared with those in the corm piece may

*The germination rate index is the mean fraction of finally emerging plants taken over several times.

explain this phenomenon. Also, it was observed that the apical bud was larger than any one of those found on the corm piece ; this might also have affected.

Establishment of plants

TABLE II

Number and percentage of stand of plants

Treatment	Establishment of plants	
	Total number	Per cent
American *dasheen*	46·0	68·0
Egyptian *dasheen*	62·6	92·0
C. D. at 5 per cent level	3·5	..
Apical piece	61·8	91·0
Cormel	60·1	88·0
Corm piece	41·1	61·0
C. D. at 5 per cent level	4·34	..

Data presented in Table II shows that the Egyptian *dasheen* was better established than the American. The germination capacity, i.e., percentage seed germination regardless of time, was 68 and 92 for American and Egyptian *dasheen* respectively. The reasons for this are not clear. However, the mucilaginous substance which is present in the Egyptian *dasheen* in larger amounts than in the American types, may give a sort of protection against the unfavourable conditions for growth.

Table II shows that the establishment of corm piece was significantly inferior to cormel and apical piece. The germination capacity was 61, 91 and 88 per cent for corm piece, apical piece and cormels in the same order. This difference might be due to relatively large cut surface liable to rot organisms. Besides, the buds on the corm piece were observed to be smaller in size than those on apical piece.

Number of corms and cormels

TABLE III

The number of cormels and corms of different types of seed and dasheen

Type of *dasheen*	Apical piece	Cormel	Corm piece	Average
Egyptian	111·5	172·7	202·7	162·3
American	577·7	515·5	312·2	468·4
Average	344·6	344·1	257·4	..

C. D. at 5 per cent level for types of seeds, 22·3

C. D. at 5 per cent level for types of *dasheen*, 16·06

C. D. at 5 per cent level for types of *dasheen* and types of seed interaction, 31·7

It is clear from Table III that the American *dasheen* produced more cormels than the Egyptian. This phenomenon shows that the apical buds of different types of *dasheen* vary in the extent of development of the lateral ones.

In general, the number of cormels produced from the apical piece treatment is more than those of the corm piece treatment.

It was found that the number of cormels produced from different types of corm piece was the least when the corm piece was used while highest in the case of the apical piece in American *dasheen*. However, the apical piece gave the smallest number of cormels in the Egyptian. No significant difference between corm piece and cormel treatments of Egyptian *dasheen* was found.

Total yield

TABLE IV

Total yield of dasheen *in tons per* feddan

Type of *dasheen*	Type of seed			
	Apical piece	Cormel	Corm piece	Average
American	15·22	14·60	9·07	12·96
Egyptian	12·67	12·76	11·01	12·14
Average	13·94	13·68	10·04	..

C. D. at 5 per cent level for types of seeds, 1·22

C. D. at 5 per cent level for types of *dasheen*, 0·99

C. D. at 5 per cent level for types of *dasheen* and types of seeds interaction, 1·72

The data presented in Table IV shows that similar yields were obtained for the two types of *dasheen*.

The apical piece and cormel treatments outyielded corm piece treatment because of the low establishment and late emergence of corm piece treatment. This agrees with Mahmoud's and Young's results. It was observed that apical piece and cormel gave strong plants.

Corm piece treatment of Egyptian *dasheen* gave bigger yields compared with American *dasheen* because of the late emergence and low establishment of the latter. Apical piece and cormel treatments of American *dasheen* outyielded similar treatments of the Egyptian.

Summary

1. Egyptian *dasheen* germinated before the American.
2. Cormel and apical piece germinated before corm piece.
3. The germination capacity was 61, 91 and 88 per cent for corm piece, apical piece and cormel respectively while taken together it was 62 and 92 per cent for American and Egyptian *dasheens* respectively. This means that types of *dasheen* and types of corm pieces have an effect on the establishment of plants.
4. No difference was found in the yield of Egyptian and American.
5. Apical piece and cormel gave a significantly higher yield than corm piece.
6. Corm piece of Egyptian *dasheen* outyielded that of American *dasheen*. Apical piece and cormel of American *dasheen* gave a bigger yield compared with similar pieces of Egyptian *dasheen*.
7. American *dasheen* produced larger number of cormels than the Egyptian.

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CARBOHYDRATE CHANGES DURING GERMINATION OF *VICIA FABA* SEEDS

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(Received for publication on 23rd December 1954)

(With 10 text-figures)

IT has been known for a long time that during early stages of germination, the growth of embryo takes place at the expense of reserves stored in the cotyledons or other storage organs. These reserves are mainly starch, protein, fat and in some seeds complex carbohydrates such as cellulose and hemicellulose. During germination there is actually a loss in the dry weight, although there may be a considerable increase in bulk and weight owing to water absorption. This loss is ascribed to respiration. As soon as germination starts a series of complex biochemical processes take place.

Detmer [1880], summarized the work done by himself and others and showed the general facts in regard to appearance, in various parts of the embryo, of starch sugar and nitrogenous compounds following the breaking down of reserved substances. Choate and Helen [1912], found that in Marquis wheat, dextrine appeared in the embryo after about 10 hours. Reducing sugars appeared in the embryo, after 18 hours and were found in a considerable quantity especially in the root hair region and coleoptile. Similar results were reported by Yocum [1925]. Tool [1924] found that during the germination of maize, reducing sugars appeared in the enlarging cells and was found in all growing parts, while non-reducing sugars were present in all parts of the embryo only during all stages. Stiles and Leach [1932] followed the course of respiration of the seeds of *Lathyrus odoratus* and showed that this course was not simple but exhibited a series of phases corresponding to the various phases of germination. James [1940] showed that during the germination of barely seeds, the changes in the trend of carbohydrate metabolism were more clearly and rapidly reflected in the content of sucrose than that of any other individual sugar. Von Ohlen [1931], dealing with soybean seeds showed that during their germination in the darkness, starch incrased in the cotyledons at first and remained fairly stable before disappearing. Reducing sugars, together with an increase in starch were noticed at first in the hypocotyl.

The aim of this work is to study the carbohydrate changes during germination of the seeds of *Vicia faba* until an increase in their initial dry weight is reached.

Materials and Methods

The dry weight as well as the air dry weight of seeds were determined. The weight of the testas of seeds was excluded from the initial weight. Twenty samples each of 10 seeds, were prepared. The seeds were then sown in pots filled with a

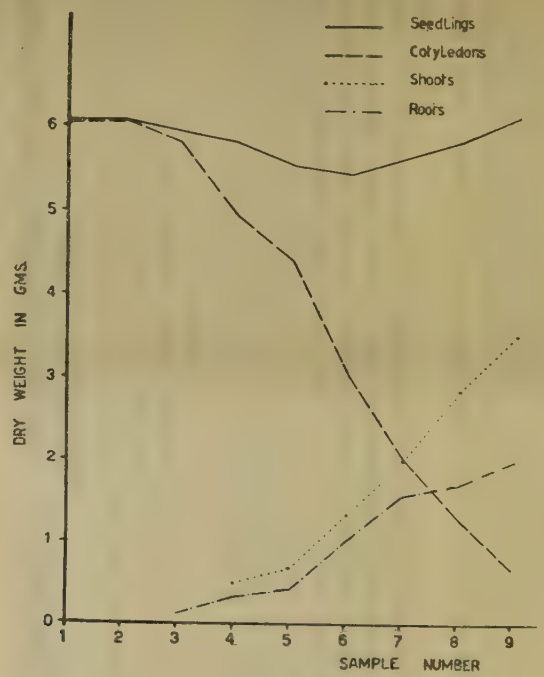

FIG. 1 Dry weight in grams of the different parts of 10 initial seeds

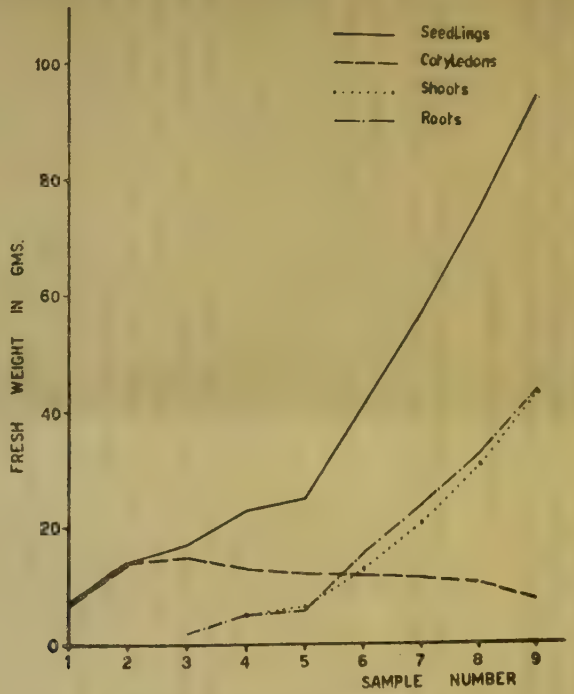

FIG. 2 Fresh weight in grams of the different parts of 10 initial seeds

mixture of 1 : 1 clay and pure acid-washed sand. The pots were manured according to the method of Gregory and Richards [1929]. Only one-third of the amounts of fertilizers were applied immediately after germination. The plants were always adequately irrigated and were left to grow under normal conditions. Samples were taken alternate days by digging the plants carefully, testas were removed, the fresh and dry weights as well as the carbohydrate fractions were determined. When it was possible to differentiate the seedlings into shoots, cotyledons and roots, it was done so.

For the determination of the different carbohydrate fractions, the methods standardised by Maskell and co-workers at the Botany School of Cambridge were used. A brief resume of the technique used for extraction, sugar analysis and determination of polysaccharides was given by Said [1941, 1945]. The sugars estimated were the sucrose as well as the free hexose sugars after taking into consideration the soluble reducing non-fermentable matter [Gawadi 1947].

Results and Discussion

Dry weight

Fig. 1 represents the dry weight in grams of the different parts of 10 initial seeds. It can be seen that there was a quick and steep decline in the initial dry weight of cotyledons, while shoots and roots showed a marked and continuous increase until the dry weight of whole seedlings exceeded their initial dry weight. The decrease in the dry weight of cotyledons showed that stored reserves were utilized in respiration and were also translocated to the newly formed organs after being transformed into simpler forms. The increase in the dry weight of shoots is due to the increasing number of leaves brought from the activity of meristematic short tips, and the stored food material translocated from cotyledons and later on from the photosynthetic activity and other anabolic processes of green cells. The increase in the dry matter of roots is due to the increasing number of its cells as well as to the food material translocated from the cotyledons and shoots.

Fresh weight

The drifts representing the fresh weight in grams of different parts of 10 initial seeds are shown in Fig. 2. It is clear from the figure that the fresh weight of cotyledons increased in the first two days. This is due to the absorption of an adequate amount of water by imbibition. In the next 48 hours, there was a slight increase in the fresh weight, followed by a slow but steady decrease which may be explained by the translocation of soluble products of reserves from the cotyledons to the growing parts of the seedling as well as their utilization in respiration, causing less concentration of the osmotically active substances present in the cotyledons and thereby resulting in less succulence. Shoots and roots showed a continuous increase in their fresh weights due to increasing number of cells. In the case of whole seedlings the increase in fresh weight is the result of the behaviour of cotyledons, shoots and roots towards succulence, causing a continuous increase.

FIG. 3 Soluble carbohydrates of the cotyledons in mg. glucose per 10 initial seeds

FIG. 4 Polysaccharides contents of the cotyledons in mg. glucose per 10 initial seeds

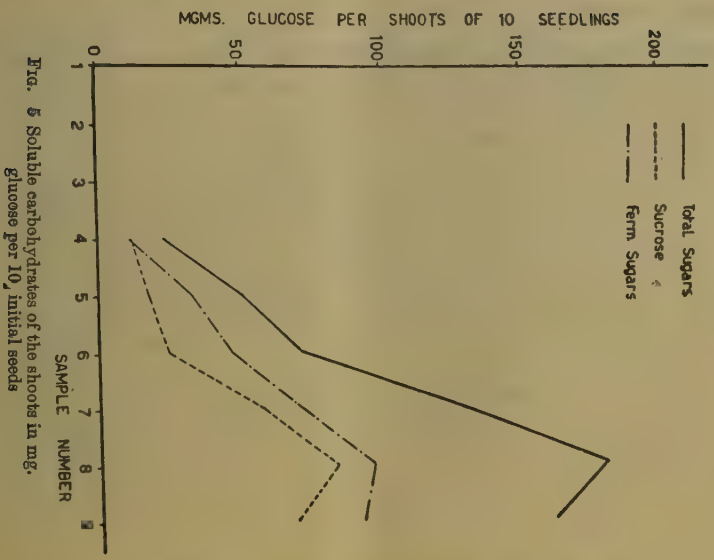

Fig. 5 Soluble carbohydrates of the shoots in mg. glucose per 10 initial seeds

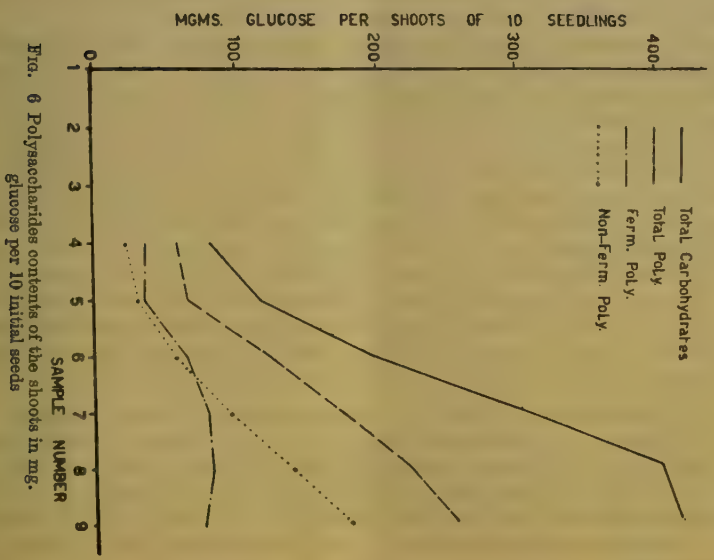

Fig. 6 Polysaccharides contents of the shoots in mg. glucose per 10 initial seeds

Carbohydrate contents

Cotyledons : (i) *Soluble carbohydrate*—The data of the soluble carbohydrate fractions of cotyledons, calculated in terms of mg. glucose per 10 initial seeds plotted in Fig. 3 revealed the fact that sucrose was taking an important part than fermentable reducing sugars. These results agree with the findings of James [1940]. Sucrose appeared in the first four samples at a more or less constant concentration, increased steadily to reach its maximum in the sixth sample after which it dropped to the last sample to reach its minimum. These results may indicate that in the first four samples the rate of hydrolysis of reserves into sucrose is more or less equal to the rate of its utilization in respiration and its translocation to the newly formed organs. From sample No. 4 to No. 6 the rate of hydrolysis exceeded that of its utilization and translocation. From sample No. 6 to the last sample the case was the reverse. Reducing sugar appeared at a very low concentration and remained fairly stable.

(ii) *Polysaccharides*—The drifts in the polysaccharides contents of cotyledons, calculated as mg. glucose per 10 initial seeds, given in Fig. 4 revealed that starch decreased slightly two days after germination, then increased two days after to a maximum value which was higher than its initial concentration, and again decreased steadily to reach its minimum in the last sample. These results agree with those of other workers, Stiles and Leach [1932] and Von Ohlen [1931]. Non-fermentable polysaccharides were not playing an important part in the cotyledons, being at a very low concentration.

Shoots : (i) *Soluble carbohydrates*—Fig. 5 represents the drifts in sugars of shoots calculated in mg. glucose per 10 initial seeds. It shows that sucrose appeared at a low concentration in the first sample (6 days after sowing) and increased to reach its maximum after 14 days of germination, followed by a slight drop to the last sample. The increase in sucrose is due to its translocation from the cotyledons as well as its formation as a photosynthetic product in later stages. James [1940] suggested that sugars from the reserves in the endosperm of germinating barley seeds enter the embryo largely in the form of sucrose. In *Vicia*, the rate of translocation of sucrose must have been at its maximum from sample No. 4 to sample No. 8, only when cotyledons contained the highest concentration of sucrose. The drop in sucrose content followed sample No. 8 corresponded to the sudden decrease of sucrose in the cotyledons. Reducing sugars behaved more or less the same as sucrose, only they differed in magnitudes being always well above those of sucrose except in sample No. 1.

(ii) *Polysaccharides*—The data of polysaccharides of shoots, calculated in terms of mg. glucose per 10 initial seeds given in Fig. 6, showed that fermentable polysaccharides did not change much in the first two samples and increased from sample No. 5 to No. 8, followed by a slight decrease to the last sample. Non-fermentable polysaccharides continued to increase from the first to the last sample, reaching a maximum concentration much higher than that reached by fermentable polysaccharides.

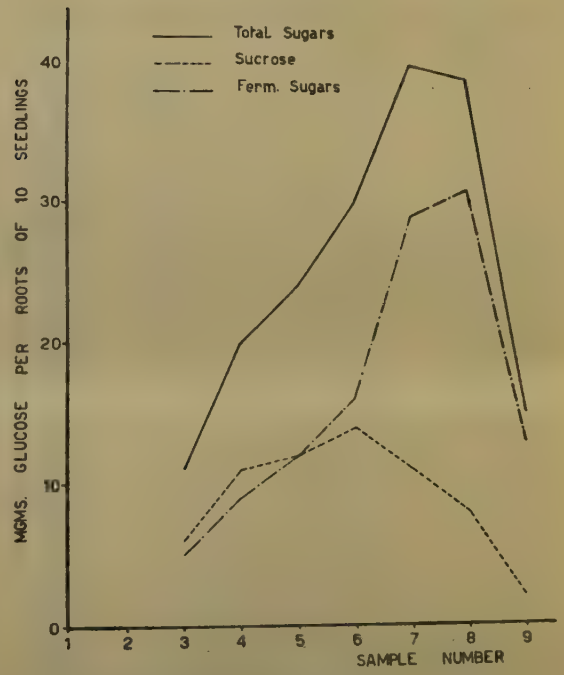

FIG. 7 Soluble carbohydrates of the roots in mg. glucose per 10 initial seeds

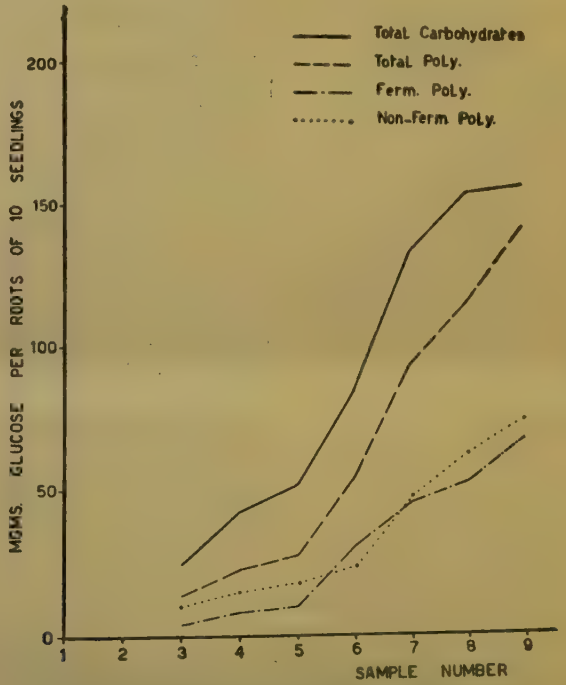

FIG. 8 Polysaccharides contents of the roots in mg. glucose per 10 initial seeds

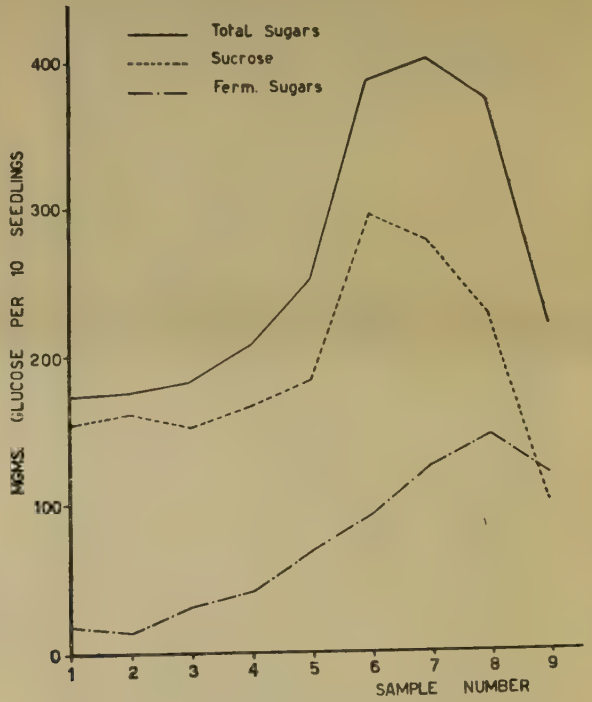

FIG. 9 Soluble carbohydrates of the whole seedlings in mg. glucose per 10 initial seeds

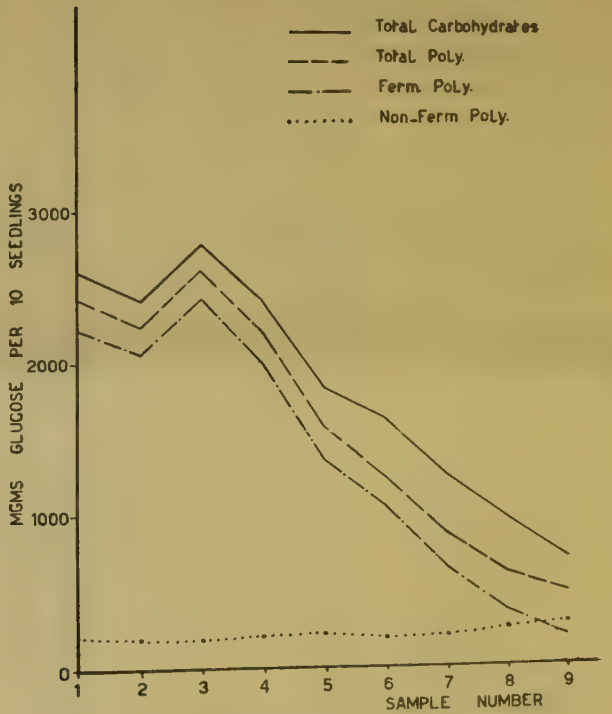

FIG. 10 Polysaccharides contents of the whole seedlings in mg. glucose per 10 initial seeds

The accumulation of polysaccharides in the shoots shows the condensation of the translocated soluble carbohydrates brought from the hydrolysis of the polysaccharides of the cotyledons as well as the products of photosynthesis of the shoots and leaves.

Roots: (i) *Soluble carbohydrates*—The drifts representing soluble carbohydrates of roots given in Fig. 7, calculated as mg. glucose per 10 initial seed, revealed that sucrose appeared in the roots at a very low concentration continued to increase until it reached its maximum in sample No. 6, after which it decreased steadily to the last sample. Reducing sugars appeared also at a very low concentration then increased steadily to reach its maximum in sample No. 8, followed by a steep decline to the last sample. According to Yocume [1925], the accumulation of these sugars in roots was more rapid than their use in the first stage when there was an adequate supply in the cotyledons. The drop followed reflected the scarcity of starch in the cotyledons of corresponding samples.

(ii) *Polysaccharides*—The drifts in the polysaccharides of roots, calculated as mg. glucose per 10 initial seeds are plotted in Fig. 8. It is seen from the figure that fermentable polysaccharides appeared at a low concentration in the first sample (sample No. 3 of germination), increased slowly till sample No. 5 and steeply to the last sample. Non-fermentable polysaccharides behaved more or less the same.

Whole seedlings: (i) *Soluble carbohydrates*—The drifts representing soluble carbohydrates in the whole seedlings calculated in mg. glucose per 10 initial seeds are shown graphically in Fig. 9. The figure shows that sucrose remained more or less constant in the first two samples, increased slowly upto sample No. 5 and steeply in sample No. 6 to reach its maximum, then decreased steadily upto sample No. 8 and steeply in sample No. 9. The behaviour of sucrose in the whole seedlings reflects its changes in cotyledons, shoots and roots, but it resembles to a great extent that of cotyledons since they contain the main food reserves. Hexoses, on the other hand increased steadily till sample No. 8, then decreased slightly to the last sample. The increase in hexoses is probably due to the accumulation of some of the products of hydrolysis of reserved food material as well as the addition of some of the products of photosynthesis.

(ii) *Polysaccharides*—The data of polysaccharides of whole seedlings calculated in mg. glucose per 10 initial seeds are given in Fig. 10. It is clearly seen from the figure that starch decreased slightly from sample No. 1 to sample No. 2, increased to a maximum in the next sample and decreased steadily upto the last sample. The increase of starch in sample No. 3 has been explained and discussed before in the results relating to cotyledons. The decrease followed is the result of the fermentable polysaccharide contents of cotyledons, shoots and roots. Non-fermentable polysaccharides showed a relatively lower value upto sample No. 7, then increased slightly to the last sample.

SUMMARY

1. Twenty samples each containing 10 seeds of *Vicia faba* were sown in plots filled with a mixture of clay and sand 1 : 1 and were irrigated and manured. Samples were collected on alternate days.

2. The dry weight and the fresh weight were studied and the results agree with the true criterion of growth.

3. Carbohydrate contents of the different parts of seedlings were studied during germination. Results showed that in cotyledons sucrose was taking an important part of the soluble carbohydrate contents. Fermentable polysaccharides showed an increase on the fourth day of germination while non-fermentable polysaccharides were not playing an important role.

4. In the shoots the translocation and accumulation of sucrose were at their maximum when the cotyledons contained the highest concentration of this sugar.

5. Roots showed a rapid accumulation of sugars in the first stage when there was an adequate supply in the cotyledons. Reducing sugars were taking a more important part than sucrose.

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GROWTH AND CARBOHYDRATE CHANGES DURING FORMATION OF *VICIA FABA* SEEDS AND FRUITS

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(With 12 text-figures)

THERE is sufficient evidence that a series of complicated morphological and biochemical changes occur during the ripening of fruits and seeds. Ripening of seeds in most plants is accompanied by gradual desiccation until they attain an air-dry state. At the same time, the dry weight increases rapidly while the water content decreases continually. Ripening of the seeds represents the embryo beginning from the fertilized egg and continuing till it becomes a small plantlet with its basic embryonic organs. Food reserves which are necessary in the first stage of germination accumulate in the seeds representing somewhat the reverse of the processes occuring during germination.

Korniche and Werner [1884], and Korniche [1908], reviewed the earlier work done in this field in Germany and concluded that the percentage of nitrogenous and mineral matter in the wheat kernel diminished as the plant matured while the percentage of carbohydrates increased, but that the absolute amount of all these constituents increased in total by maturity. Brenchley and Hall [1908-1910], distinguished three stages in the formation of wheat grown : (*a*) a period during which the nitrogen percentage of the grain falls rapidly while both the dextrose and the diastatic power rise, (*b*) a period during which the endosperm is filled and the dry weight more than trebled, and (*c*) the ripening period which is characterised by the desiccation of the grain, but when simultaneously the maximum dry weight is reached. Brenchley [1912], noticed that in the grains of barley the percentage of dextrose in the dry matter showed a slight fall in the beginning and then remained constant to the very end. Thatcher [1913], found that during the development of the wheat kernel, true starch was very low at first but rose rapidly as the endosperm filled. He also showed that the percentage of sugars decreased much more rapidly during the early stages than after the " milk stage ", no doubt by reason of their conversion into reserve starch, after which, the decrease was very slight. Pickett [1950], dealing with peanut seed found that during the two weeks preceding maturity, fat and protein increased considerably on a weight per seed basis. The ratio of protein : carbohydrate, fat : carbohydrate and fat : protein increased as the seeds developed. The same author noticed that the percentage of reducing sugars, sucrose and starch decreased during the early stages of growth while sucrose increased near maturity so that the total percentage of sugars and starch approached a constant value in the fortnight preceding maturity. He also showed that during the development of the shell the percentage of dry matter, fat, protein, sugars and starch decreased while hemicellulose increased.

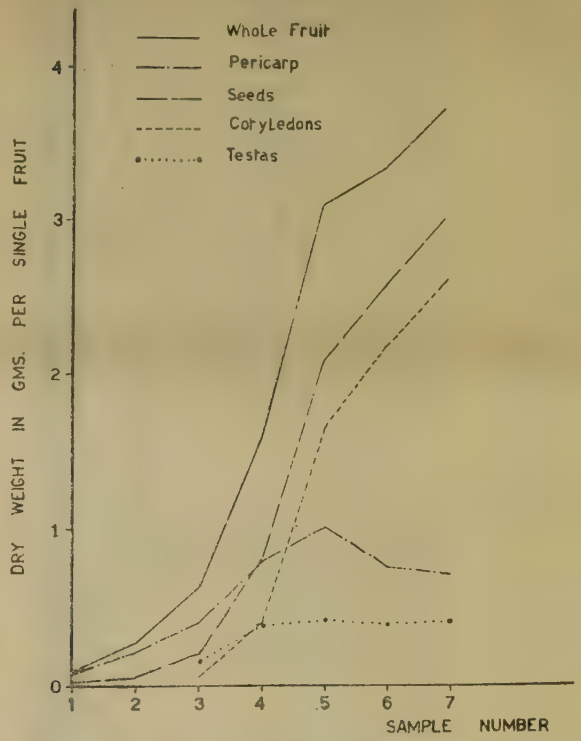

Fig. 1. Dry matter in grams of the different parts of one fruit

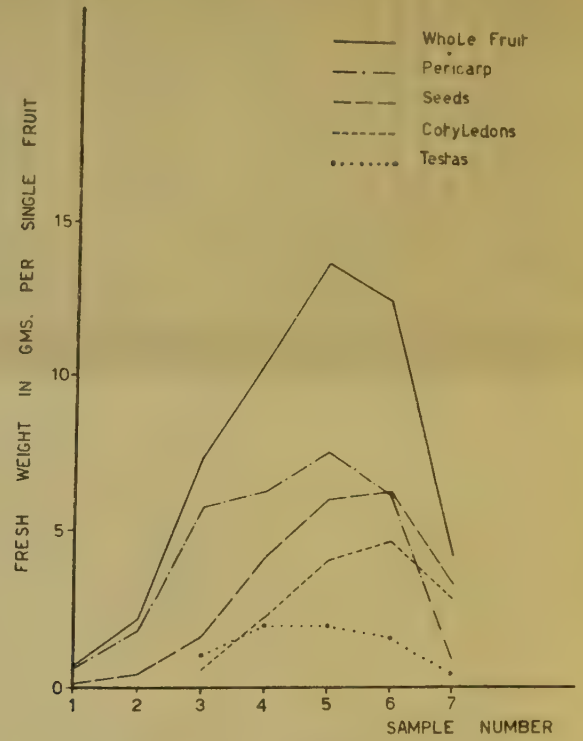

Fig. 2. Fresh weight in grams of the different parts of one fruit

The authors conducted this work to study the carbohydrate changes taking place during the growth and development of the seeds and fruits of *Vicia faba*.

Materials and Methods

A piece of land of 20 square meters (10×2 meters) area on the Experimental Fields of the Faculty of Agriculture, was chosen to carry out this experiment. The application of manuring, irrigation and other field treatments were normal.

The first fruit samples were taken 15 days after fruit setting, and the rest of the samples at 15 days interval until the seeds were mature and harvested. The fruit samples collected at every time of sampling were divided into two comparable lots. Each fruit was separated, wherever possible, into pericarp, cotyledons and testas. The fruits of the first lot were used for determination of the fresh weights and dry weights, while the fruits of the second lot were used for determination of the different fractions of carbohydrates, according to the methods standardized by Maskell and co-workers at the Botany School of Cambridge. A brief resume of the technique used for extraction, sugar analysis and the determination of polysaccharides was given by Said [1941, 1945]. The sugars estimated, after taking into consideration the soluble reducing non-fermentable matter, were the sucrose and the true hexose sugars [Gawadi, 1947].

Results and Discussion

Dry weight

The data of the dry matter in grams of the different parts of one fruit are shown in Fig. 1. It shows that there was a quick and steep increase in the dry weight of cotyledons. This is interesting since it shows the accumulation of the food reserves in the cotyledons. The dry weight of the testas showed a marked increase in the first three samples, followed by a slight increase in the next three samples and remained fairly stable to the last. The dry weight of the whole seeds increased slowly in the first three samples, then steeply to the last. This also is interesting since it shows the accumulation of the reserves during the ripening of seeds which represents somewhat the reverse of the process occuring during germination. These results agree with those of Brenchley and Hall [1942]. The dry weight of the pericarp increased steadily till sample no. 5 to reach its maximum, then a continuous decrease followed to the last sample. The increase in the dry weight of the pericarp indicates the accumulation of some food reserves in its tissues as well as other growth phases including cell divisions and cell enlargement. The drop followed is due to translocation of some of the food reserves to other parts of the fruit and also to the utilization of some of these reserves in respiration. These results again agree with those reported by Pickett [1950].

Fresh weight

The drifts representing the fresh weight in grams of the different parts of one fruit are given in Fig. 2. The figure shows that the fresh weight of the cotyledons increased rapidly from sample no. 3 to sample no. 6 to reach its maximum, and

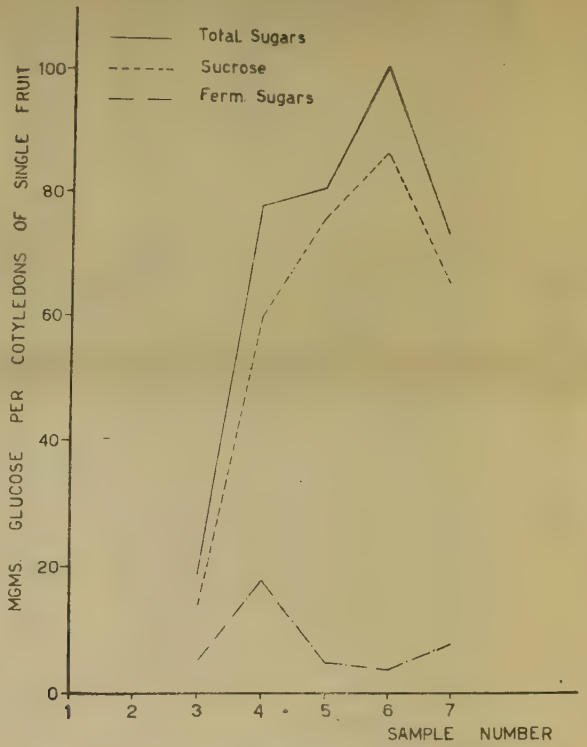

FIG. 3. Sugars in the cotyledons of one fruit in mgms. glucose

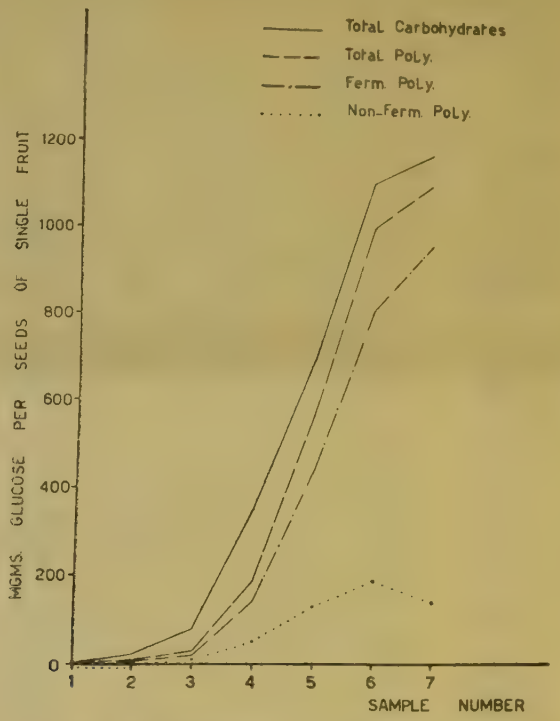

FIG. 4. Polysaccharides in the cotyledons of one fruit in mgms. glucose

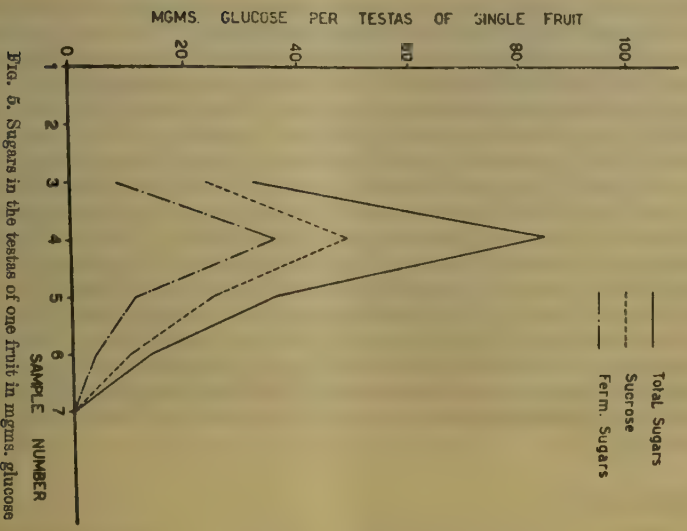

Fig. 5. Sugars in the testas of one fruit in mgms. glucose

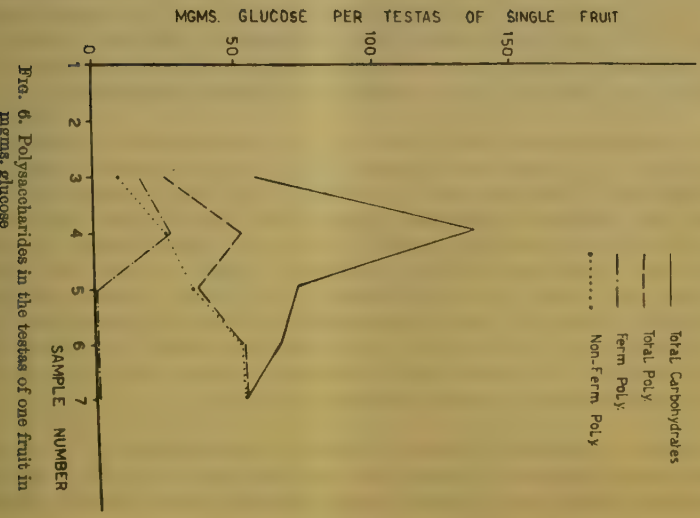

Fig. 6. Polysaccharides in the testas of one fruit in mgms. glucose

then decreased. Testas, increased in sample no. 3 and sample no. 4, remained fairly stable in the next sample and then decreased steadily to the last. In the case of whole seeds, the fresh weight increased slowly and steadily in the first three samples, then steeply to the fifth, followed by a slight increase in sample no. 6 to reach its maximum, and then dropped to the last sample. The increase in the fresh weight of cotyledons, testas and whole seeds in the first stage of seed formation reflects the accumulation of osmotically active substances causing absorption of adequate amounts of water. The drop followed is due to the transformation of these substances into insoluble or almost insoluble substances in the later stages causing the desiccation of the seeds [Brenchely and Hall, 1908-10]. The fresh weight of the pericarp increased steadily till sample no. 3, then slowly to sample no. 5 reaching a maximum value, after which it decreased slowly in the next sample and steeply in the subsequent ones. The increase indicated the accumulation of osmotically active substances during the first stage. The drop followed may be explained as the translocation of those soluble reserves to other parts of the fruit—mainly seeds —causing the desiccation of the pericarp. The behaviour of the fresh weight of the whole fruit reflected the changes in the fresh weights of the cotyledons, testas and pericarp.

Carbohydrate contents

Cotyledons : (i) *Sugars.* —The value of sugars of the cotyledon calculated in terms of mg. glucose per cotyledon of single fruit given in Fig. 3 show that sucrose appeared at a low concentration, increased rapidly till sample no. 6 to reach a maximum, followed by a drop to the last sample. These results show that sucrose is the principal sugar in the cotyledons of *Vicia*, and its increase indicates its translocation and accumulation in the cotyledons, while the decrease followed may indicate its transformation into other complex reserves as well as its utilization in respiration. Reducing sugars seemed of no significant importance.

(ii) *Polysaccharides.*—The drifts of the polysaccharides of the cotyledons calculated in mg. glucose per cotyledons of single fruit and plotted in Fig. 4 indicate that fermentable polysaccharides appeared in a very low concentration in the first sample, then increased steadily and steeply to reach its maximum in the last sample. These results are interesting since they show the accumulation and storage of fermentable polysaccharides in the cotyledons. Similar results were reported by Korniche and Werner [1884] and Korniche *et al.* [1908]. Non-fermentable polysaccharides, on the other hand, appeared also in a very low concentration, increased slowly up to sample no. 6 to reach its maximum after which it decreased slightly to the last sample. The values of non-fermentable polysaccharides were on the whole much lower than those of fermentable polysaccharides.

Testas : (i) *Sugars.*—The data of sugars in testas given in Fig. 5 and calculated in mg. glucose per testas of *Vicia* contained a considerable amount of sucrose in the first sample (sample no. 3), which increased steadily in the following sample after

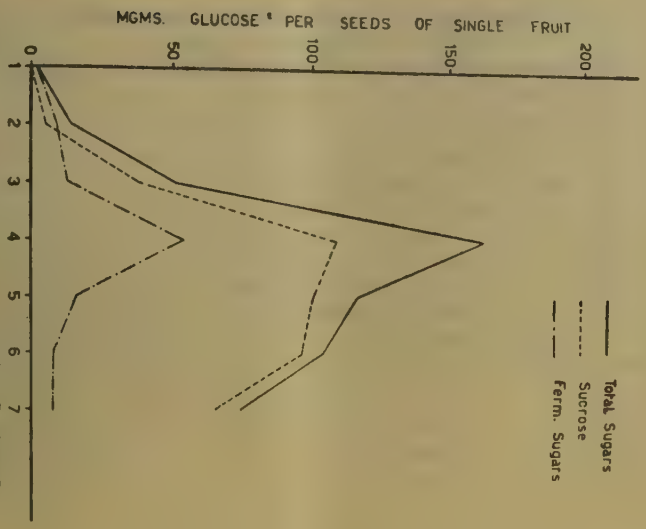

Fig. 7. Sugars in the seeds of one fruit in mgm. glucose

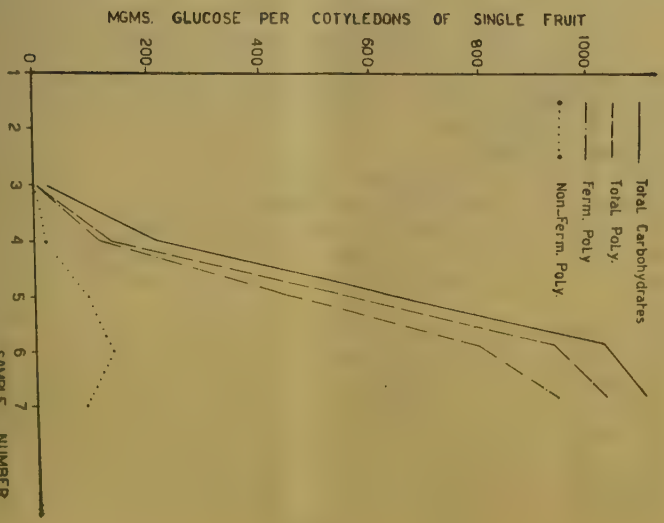

Fig. 8. Polysaccharides in the seeds of one fruit in mgm. glucose

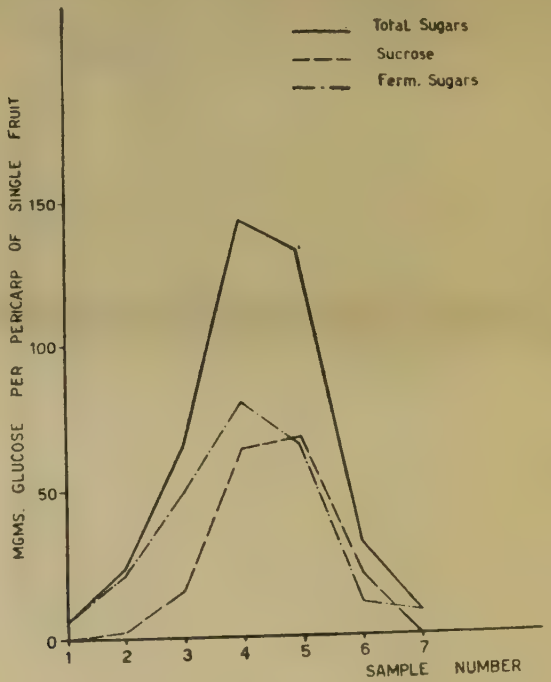

FIG. 9. Sugars in the pericarp of one fruit in mgms. glucose

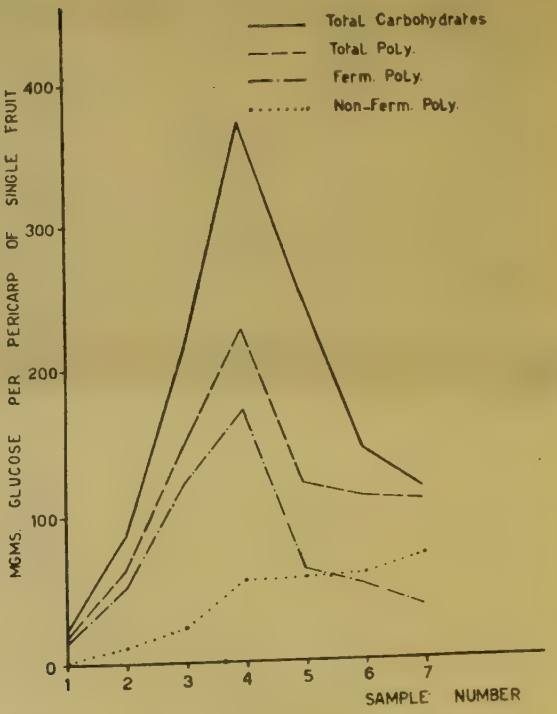

FIG. 10. Polysaccharides in the pericarp of one fruit in mgms. glucose

which it decreased steeply to the last sample. The first two samples indicated the translocation to and the accumulation of sucrose in the testas, while the following samples showed its translocation to the cotyledons and perhaps its utilization in respiration. Fermentable sugars behaved almost in the same manner as sucrose, with the difference in magnitudes being always in less amounts.

(ii) *Polysaccharides*—The values of polysaccharides of the testas calculated in mg. glucose per testas of the seeds of single fruit given in Fig. 6 show that the fermentable polysaccharides increased from sample no. 3 to sample no. 4 to reach a maximum, and then decreased steeply in the following sample recording no value in the last sample. This is interesting since the increase in fermentable polysaccharides showed their accumulation in the testas during the first stages of development. The drop followed is mainly due to the translocation of fermentable polysaccharides from the testas to the cotyledons after being hydrolysed into soluble form. Non-fermentable polysacchrides increased steadily till sample no. 6 to reach a maximum then remained fairly stable to the last sample.

Seeds : (i) *Sugars*—Fig. 7 represents the drifts in sugars of the seeds calculated in mg. glucose per seed of single fruit. It can be seen that sucrose had practically no value at first, increased slowly in the second sample, then quickly up to sample no. 4 reaching its maximum concentration. From sample no. 4 to 6 there was a slight decrease followed by a sudden drop in the last sample. The behaviour of fermentable sugars on the whole resembled that of sucrose but they only differed in magnitudes. The increase of these sugars indicated their accumulation in the seeds and also showed that the rate of their translocation and accumulation was much greater in that stage than their utilization in respiration as well as of their transformation into other complex food reserves.

(ii) *Polysaccharides*—The drifts of polysaccharides contents of the seeds given in Fig. 8 and calculated in mg. glucose per seed of one fruit, indicate that fermentable polysaccharides had practically no value, increased slightly till sample no. 3, then quickly and steeply up to the last sample. The slow increase noticed at first indicated that the rate of starch synthesis was much faster. Similar results were reported by Thatcher [1913]. Non-fermentable polysaccharides also showed very little value in the first three samples, increased steadily up to sample no. 6 and was followed by a slight decline to the last sample. The values of non-fermentable polysaccharides were on the whole much lower than those of fermentable polysaccharides.

Pericarp : (i) *Sugars*—Fig. 9 represents the drifts in sugars of the pericarp calculated in mg. glucose per pericarp of single fruit. It is clear from the figure that sucrose had negligible value in the first two samples, increased steadily to reach its maximum in sample no. 5 after which it decreased steeply up to the last sample when it showed no value. Fermentable sugars behaved almost like sucrose, only its maximum concentration was reached in the fourth sample. These results show that there were two significant phases taking place during the growth and development of the pericarp. The first phase is characterised by the accumulation

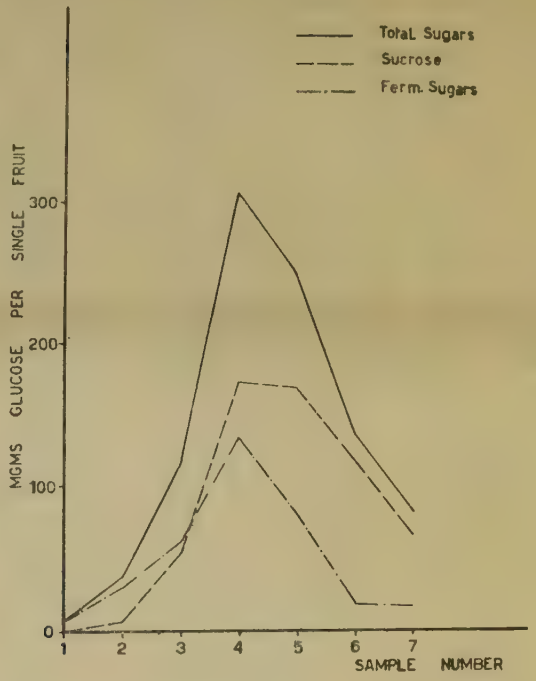

FIG. 11. Sugars in the whole fruit in mgms. glucose

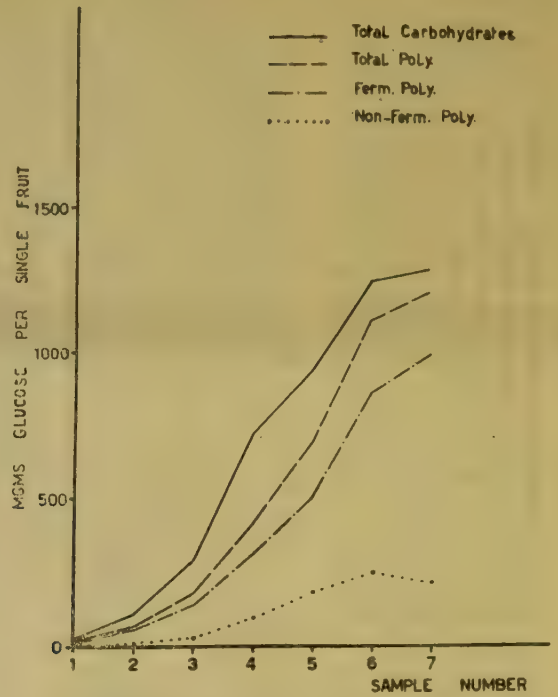

FIG. 12. Polysaccharides in the whole fruit in mgms. glucose

of sugars while the second phase is characterised by the disappearance of these sugars, probably due to their translocation and storage in the growing seeds as well as their utilization in respiration and other metabolic processes. Pickett [1950] reported similar results on the shells of peanut.

(ii) *Polysaccharides*—The drifts of polysaccharides of the pericarp represented graphically in Fig. 10 and calculated in mg. glucose per pericarp of one fruit showed that fermentable polysaccharides behaved more or less like sugars. Here again one can see the two phases previously mentioned in sugars. Non-fermentable polysaccharides, on the other hand, behaved differently as they increased gradually up to the last sample. In this connection it may be mentioned that Pickett [1950] working on the shells of peanut reported similar conclusions.

Whole fruit: (i) *Sugars*—The data of sugars in whole fruit given in Fig. 11 and calculated in glucose per fruit indicate that sucrose showed no value in the first sample, increased slowly in the next sample quickly up to sample no. 4 to reach a maximum, remained fairly stable in the next sample and then suddenly decreased up to the last sample. The trend of fermentable sugars resembled to a great extent that of sucrose. The two phases mentioned before can also be noticed here. The first phase, starting from sample no. 1 to sample no. 4 is characterised by the accumulation of sugars due to photosynthetic activity of the green cells of fruits as well as to the transformation of these sugars from other organs. In this phase it seems that the rate of transformation and accumulation of sugars was much greater than their utilization and transformation into other complex food and also to their utilization in respiration.

(ii) *Polysaccharides*—Fig. 12 shows the drifts in polysaccharides of the whole fruit, calculated in mg. glucose per fruit. It can be clearly seen from the figure that fermentable polysaccharides increased slowly in sample 2, then steeply up to the last sample to reach its maximum. These results show a continuous accumulation of starch as the fruits proceed to maturity. It also shows that the rate of starch synthesis was not great in the first two samples and was accelerated in the following samples. Non-fermentable polysaccharides appeared in a relatively lower concentration, increased steadily till sample no. 6 and decreased slightly up to the last sample.

Summary

1. Seeds of *Vicia faba* were sown in the experimental fields of the Faculty of Agriculture, Giza, and were left to grow under normal conditions. First fruit samples were taken 15 days after setting and the rest of the samples collected at 15 days interval.

2. The fruit collected at every time of sampling were separated into pericarps, cotyledons and testas when it was possible to do so.

3. The dry weight and fresh weight were studied and the results of dry weight agreed with the true criterion of growth.

4. Carbohydrate contents of different parts of the fruit were studied. Results showed the continuous accretion and storage of carbohydrates in the cotyledons. Sucrose was the principal sugar in the cotyledons.

5. Carbohydrates accumulated in the testas during the first stage after which sugars and starch disappeared.

6. The behaviour of carbohydrates in the pericarp of fruit showed that there were two phases taking place during its growth and development. The first phase is characterised by the accumulation of carbohydrates in its tissues, while in the second phase the case was reverse probably due to translocation of these carbohydrates to the growing seeds.

7. In the case of whole fruit, sugars showed two significant phases, while fermentable polysaccharides showed a continuous increase.

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PERSISTENCE OF YELLOW-VEIN MOSAIC VIRUS OF *ABELMOSCHUS ESCULENTUS* (L.) MOENCH IN ITS VECTOR *BEMISIA TABACI* (GEN.)

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YELLOW-VEIN mosaic of *Abelmoschus esculentus* (L.) Moench [*Ocrovena hibiscae*, Capoor and Varma, 1950] cannot be transmitted by sap but is readily transmitted by the white-fly, *Bemisia tabaci* Gen. It is a persistent virus and undergoes a minimum latent period of six to eight hours in the vector. Also the white-flies after feeding for 24 hours on diseased plants remain viruliferous throughout their life span of about 24 days [Varma, 1952]. Most of the experiments to determine the retention of the plant viruses by insect vectors have been carried out with leaf hoppers and not much information is available about the white-fly and the viruses it transmits. Experiments were, therefore, conducted to determine the number of days for which the white-fly remained infective after feeding for varying periods on diseased plants.

Material and Methods

White-flies for experimental work were obtained from virus-free colonies of the insect reared under insect-proof conditions. Diseased plants used as a source of virus were those that had been diseased for over two months and exhibited typical vein mosaic symptoms. Test seedlings were raised in four-inch clay pots and inoculated when they were three to four weeks old.

Since the male white-flies are very short-lived, freshly emerged female white-flies were employed for these tests. The insects were subjected to a 'Preliminary Fasting' for four hours followed by 'Infection Feeding' for periods varying from 30 minutes to 24 hours. The insects were confined in micro-cages singly and fed on the lower surface of the second leaf (counting from the top) of the test plant. The micro-cages of white-flies which fed on diseased plants for more than two hours were

connected to vacuum pump [Varma, 1952]. Thus they lived longer but this in no way vitiated the results of the experiment. Each white-fly was transferred after 24 hours to a fresh healthy seedling and this was continued as long as it survived. For each feeding period 30 to 40 flies were tested. All test plants were kept under observation for 60 days. The inoculated leaf from each test plant was clipped off after 10 days.

Experimental

(*a*) *Number of white-flies becoming infective at the end of different ' Infection Feeding ' periods*

The total number of white-flies tested and those that proved infective after different ' Infection Feeding ' periods are shown below :—

Infection Feeding period	No. of insects tested	No. of infective insects	Percentage
30 minutes	30	14	35·8
1 hour	40	25	62·5
2 hours	38	28	73·6
4 hours	32	23	71·8
6 hours	30	21	70·0
24 hours	63	48	76·1

Out of the white-flies which proved infective, some died within a few days and others escaped or were crushed accidentally. The transmission records of such insects have therefore not been included.

(*b*) *Transmission of the virus by white-flies given ' Infection Feeding ' for different periods*

The detailed transmission records of white-flies tested for ' Infection Feeding ' periods of 30 minutes, 1 hour and 2 hours as also for 4 and 6 hours are set out in Tables I and II respectively.

TABLE I

Persistence of virus in individual white-flies given different 'Infection Feeding' periods

Infection Feeding period	Serial No. of flies	Infection produced after days																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
30 minutes	1	−	+	−	−	−	−	−	−	−	−	−	−	D							
	2	+	−	−	−	−	−	−	−	−	−	−	D								
	3	−	+	−	−	D															
	4	−	+	−	−	−	−	D													
	5	−	+	−	−	−	−	D													
	6	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	D		
	7	−	+	−	−	−	−	−	−	−		−	D								
	8	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	D				
	9	−	+	+	−	−	−	−	−	−	−	−	−	D							
	10	−	+	−	−	−	−	−	−	−	−	−	−	D							
1 hour	1	+	−	+	−	−	−	−	−	−	D										
	2	−	+	−	−	−	−	−	−	−	−	−	−	D							
	3	−	+	+	+	−	−	−	D												
	4	−	+	+	−	−	−	−	D												
	5	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	D		
	6	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	D		
	7	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	D				
	8	+	+	−	−	−	−	−	−	−	−	−	−	−	−	D					
	9	+	+	−	−	−	−	−	−	−	−	−	−	−	D						
	10	+	+	+	−	−	−	−	−	−	−	−	−	−	D						
	11	−	+	−	−	+	−	−	−	−	−	−	D								
	12	+	−	−	−	−	−	−	−	−	−	−	−	−	−	D					

TABLE I—*contd.*

Persistence of virus in individual white-flies given different 'Infection Feeding' periods

Infection Feeding period	Serial No. of flies	Infection produced after days																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 hours—*contd.*	13	+	—	—	+	—	—	—	—	—	—	—	—	D							
	14	+	—	—	—	—	—	—	—	—	—	D									
	15	+	+	—	—	—	—	—	—	—	—	—	—	—	D						
2 hours	1	—	+	+	+	+	+	—	—	D											
	2	—	+	—	+	—	—	—	—	+	—	—	—	—	—	D					
	3	—	+	+	+	+	—	—	—	—	—	—	—	D							
	4	+	+	—	—	—	D														
	5	—	+	—	—	—	—	+	—	—	+	—	—	—	—	—	D				
	6	—	+	+	+	+	—	+	—	—	+	—	—	—	—	—	—	D			
	7	+	+	+	+	+	+	—	+	—	—	—	D								
	8	+	—	—	—	+	—	—	+	—	—	—	—	—	D							
	9	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	D
	10	+	—	+	+	+	—	+	—	—	+	—	—	—	—	—	—	—	D		
	11	+	—	+	—	+	—	—	—	—	+	—	—	—	—	—	D				
	12	—	+	+	+	+	—	—	—	—	—	—	—	—	—	D					
	13	+	—	—	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	D
	14	+	+	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	D		
	15	+	—	—	—	—	+	—	—	—	—	—	—	D							
	16	+	—	—	+	—	—	—	—	—	—	—	D								
	17	+	+	—	—	—	—	—	—	—	—	D									
	18	+	—	+	+	—	—	—	—	—	—	—	—	—	—	—	D				

+ = Positive infection
— = Negative infection
D = Death of the insect

TABLE II

Persistence of the virus in individual white-flies given different 'Infection Feeding' periods

Infection Feeding period	Serial No. of white-flies	Infection produced after days																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
4 hours . .	1	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	D											
	2	+	+	+	+	—	+	+	—	+	—	+	—	+	—	+	—	+	—	—	—	D							
	3	+	+	+	+	+	+	+	D																			
	4	+	+	—	+	—	—	—	+	—	—	+	+	—	—	D													
	5	—	+	+	+	—	+	+	—	+	—	+	—	+	—	+	+	—	—	D									
	6	+	+	—	—	—	—	—	—	+	—	—	—	—	+	—	—	—	—	D									
	7	+	+	—	+	+	+	—	+	—	—	—	—	+	—	—	—	—	—	—	—	D							
	8	—	+	—	+	+	—	—	—	+	—	—	—	D															
	9	—	+	+	+	+	+	+	+	—	+	+	—	+	—	—	—	—	—	—	—	—	—	D					
	10	+	—	+	—	—	+	+	—	—	—	—	—	—	—	—	D												
	11	+	—	+	—	—	—	—	—	—	—	+	—	—	—	D													
	12	+	+	+	+	+	+	—	+	—	—	—	—	D															
	13	—	+	—	—	—	+	—	—	—	—	—	+	+	—	—	—	D											
	14	—	+	—	+	+	+	+	+	—	—	—	—	—	—	—	—	—	D										

TABLE II—*contd.*

Persistence of the virus in individual white-flies given different 'Infection Feeding' periods

Infection Feeding Period	Serial No. of White-flies	Infection produced after days																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
4 hours—*contd.*	15	+	—	—	+	+	—	—	+	—	—	—	—	D															
	16	+	—	—	+	+	+	+	—	—	+	—	—	+	—	+	—	—	D										
6 hours . .	1	—	+	+	+	+	+	+	+	+	—	+	+	+	—	+	+	—	D										
	2	+	—	+	—	—	—	—	+	—	+	+	—	—	—	—	—	—	—	+	—	—	D						
	3	—	+	+	+	+	+	+	+	+	+	—	—	+	+	+	+	+	—	+	—	—	D						
	4	+	—	—	—	—	+	—	—	+	+	—	—	—	—	—	—	—	—	D									
	5	+	+	+	+	+	+	+	+	+	—	+	+	—	+	+	+	—	+	—	+	—	+	—	—	—	D		
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	D														
	7	+	—	+	—	+	+	+	+	—	—	+	+	+	+	+	—	—	+	—	—	+	+	—	—	—	—D		
	8	+	+	—	+	+	—	+	—	—	—	+	—	—	—	+	—	D											
	9	+	—	+	+	+	+	+	+	+	+	+	—	—	—	+	—	D											
	10	—	+	+	+	+	+	+	+	—	+	+	—	+	+	+	—	D											
	11	+	+	+	—	+	+	—	+	—	—	+	+	+	+	+	+	+	+	—	—	D							
	12	+	—	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	+	—	—	—	+	—	D				
	13	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	D												
	14	—	+	—	—	—	+	+	—	—	—	—	+	—	—	—	D												
	15	+	+	—	—	+	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	D							
	16	—	+	—	—	—	+	+	+	—	—	—	+	—	—	—	—	+	—	—	+	—	—	D					

+ = Positive Infection
— = Negative Infection
D = Death of the insect

The data in Tables I and II show that out of the insects given an ' Infection Feeding ' period of 30 minutes, only one fly gave infection on two successive days while the rest were able to infect only on one day. White-flies given an 'Infection Feeding ' of one hour retained the virus up to fifth day. White-flies which were fed for two hours on diseased plants retained the virus up to tenth day.

The maximum time for which white-flies, given an ' Infection Feeding ' of four and six hours, could retain the virus was 17 and 20 days respectively. The latter period also represents the maximum limit of longevity of these insects under the condition of the experiment. Again the transmissions were more sustained and in some cases infection was obtained consecutively for 13 days.

For each of the above tests controls were kept by testing flies that had fed on diseased plants for 24 hours. These tests were continued for two years and in all 63 insects were tested. Individual white-flies varied considerably in their ability to infect healthy *bhendi* (*Abelmoschus esculentus*) plants in daily transfers and several cases were encountered in which no transmissions were obtained even after 24-hour ' Infection Feeding ' while in others the transmissions were few and erratic. The records of 20 representative flies including the most efficient as well as the weakest are given in Table III.

The transmission of *bhendi* yellow-vein mosaic virus by white-flies after ' Infection Feeding ' period of 24 hours is similar to that obtained from flies that had fed for six hours on diseased plants. Some insects were able to transmit the virus even on the 25th day just prior to their death.

Discussion

The data obtained clearly indicate that *Bemisia tabaci* is able to retain the virus of yellow-vein mosaic of *A. esculentus* throughout its life, provided it is able to acquire a sufficient charge of the virus by feeding on diseased plants for at least six hours. The insects that had been fed for shorter periods on diseased plants invariably lost infectivity after some time by continuous feeding on healthy plants. Thus the white-flies which were given an ' Infection Feeding ' for 30 minutes lost the virus within 24 hours after feeding on a healthy *bhendi* plant. A feeding period of one hour on the inoculum could keep the white-flies infective up to five days and there was a progressive increase in retention of the infectivity as the ' Infection Feeding ' period was increased from two to four hours. Some of the insects after four-hour ' Infection Feeding ' were infective up to 16 days. Maximum infectivity was obtained by prolonging the ' Infection Feeding ' period to six hours, for one of the insects so treated could retain the virus up to 25 days as those which had been fed on the inoculum for 24 hours.

Kirkpatric [1931] showed that the white-fly (*Bemisia gossypiperda*; now called *B. tabaci*) which had fed on diseased cotton plants for a sufficiently long period could infect healthy cotton plants with curl after feeding for three days on

TABLE III

Persistence of the virus in individual insects given 'Infection Feeding' for 24 hours

Serial No. of flies	Infection produced after days																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	D											
2	+	+	+	—	—	+	—	+	+	+	+	+	+	—	+	—	+	—	—		D								
3	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	—	+	+	—	+	+	+	—	—	—	D
4	—	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	—	+	+	+	—	—	+	—	—	D		
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D													
6	—	+	+	+	—	—	+	—	—	—	—	+	—	—	—	+	+	+	—	—	—	—	D						
7	+	+	+	+	+	+	—	+	+	—	+	—	+	+	+	+	—	—	D										
8	+	+	+	+	+	+	+	+	+	—	—	+	—	—	+	—	—	—	+	—	—	—	+	—	—	D			
9	—	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	—	+	—	D								
10	—	+	—	+	+	+	+	—	+	+	+	—	—	—	+	+	+	+	—	D									
11	—	+	+	+	+	+	+	+	+	+	—	—	+	—	+	+	+	+	+	D									
12	+	—	+	+	+	+	+	+	+	+	+	+	D																
13	+	+	+	+	+	+	D																						
14	+	+	+	+	+	+	—	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	—	D					
15	—	+	+	—	—	+	+	—	—	+	+	+	+	—	+	+	—	—	—	+	—	+	—	—	—	D			
16	+	+	+	+	+	+	+	+	—	+	—	+	—	—	—	—	+	—	—	+	+	—	—	D					
17	—	+	—	+	+	+	—	—	—	+	—	—	—	+	—	+	—	—	—	—	+	+	D						
18	+	+	+	+	—	+	+	+	+	+	+	—	+	+	+	—	—	—	—	+	+	—	D						
19	+	+	+	+	+	+	—	—	—	+	+	+	+	—	+	+	+	—	—	+	+	—	+	—	—	—	—	D	
20	+	—	+	+	—	+	+	+	—	—	+	+	—	—	+	+	+	+	—	—	—	+	D						

+ = Positive infection
— = Negative infection
D = Death of the insect

Dolichos lablab plants which were immune from cotton leaf curl virus. Costa and Bennett [1950] showed that *Bemisia tabaci* was able to retain the virus of *Euphorbia* mosaic for 18 days. The experiments reported in this paper show that white-fly could retain the virus of yellow-vein mosaic of *A. esculentus* for 25 days.

These results are somewhat similar to those obtained by Freitag [1936] with the beet leaf hopper, *Eutettix tenellus* and the sugar beet curly top virus (*Ruga Verrucosans*, Carsner and Bennett). Jassids fed for 10 minutes to three hours on curly top beets infected fewer plants in their lives than when the feeding period on the inoculum was increased from six hours to 28 days. Bennett and Wallace [1938] reported that non-viruliferous leaf hoppers (*Eutettix tenellus*) acquired sufficient virus in two days to impart to them maximum ability to infect seedling beets. Leaf hoppers that had acquired their charge of virus during a six-hour feeding on an extract from viruliferous leaf hoppers showed a weakening of their infective power after a time. Storey [1938] also concluded that the length of time for which jassids remained infective is in some way related to the 'Infection Feeding' period on maize plants infected with streak virus.

A possible explanation for the inability of the white-flies that had fed for 30 minutes on the inoculum, to retain the virus beyond the first day may be due to the fact that after the acquisition of the virus all of it was regurgitated before it could permeate from the alimentary canal to the salivary glands through the blood.

Summary

The virus of yellow-vein mosaic of *Abelmoschus esculentus* persists in the female white-fly (*Bemisia tabaci*, Gen.) throughout its life. A feed of four to six hours on source of inoculum may enable a white-fly to carry virus throughout its life. When the 'Infection Feeding' period is less, the white-flies may soon lose the infective power by continuous feeding on healthy plants. The ability of the white-fly to retain the virus for a long period makes it a very potent vector.

Acknowledgement

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COMPOSITION AND NUTRITIVE VALUE OF JACK FRUIT

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(Received for publication on 22nd March 1955)

JACK fruit (*Artocarpus integrifolia*) which is abundantly available, particularly in the South, has not so far been fully exploited by the fruit preservation industry. On account of the very large size of the fruit and high yields, it occupies front rank among the South Indian fruits in the quantity of food produced per unit area [Naik, 1949]. On a conservative estimate, it is presumed that there are more than 1,00,000 jack fruit trees in South India alone [Naik, *loc. cit.*]

In the course of an investigation on the utilization of this fruit for preservation in different forms, its composition and nutritive value were also studied. The chemical composition of the edible parts of ripe jack fruit has been reported by Pratt and Del Rosario [1913], Thompson [1914] and Aykroyd [1951]. These data are, however, incomplete in some respects. In this paper, the results of a more thorough analysis of the ripe as well as green jack fruit are briefly discussed.

Material and Methods

In this investigation 2909 lb. of ripe jack fruit and 435 lb. of raw fruit of assorted grades and varieties, available in the Mysore market during the main season of availability, viz., February to August, were employed. In some cases fruits of known origin were also used. For analysis, the edible portion of green jack fruit, peeled seeds, the rind and its inner portion were cut into small bits, dried in a cabinet-drier at 140°F and stored in stoppered glass bottles for subsequent analysis. The bulbs were, however, analysed in the fresh condition. Moisture, ash, ether extract, acidity, crude fibre, acid hydrolysable starch, alcohol insoluble solids and calcium were determined by A. O. A. C. methods [1950]; protein by microkjeldahl method [Belcher and Godbert, 1945]; sugars in the alcohol soluble solids by volumetric method of Lane and Eynon [1923]; phosphorus by the method of Fiske and Subba Row [1925]; iron by the method of Wong [1928]; carotene by chromatographic method of the Association of Vitamin Chemists [1951] using 0·025 per cent potassium dichromate as test standard [Seaber, 1940]; ascorbic acid by direct titration of the extract in metaphosphoric acid with 2-6 dichlorophenol-indophenol; thiamine by thiochrome method (Wang and Harris, 1940] and pectin by the method of Carre and Haynes (1922).

Results

The average composition of different parts of the ripe jack fruit based on the analysis of 126 fruits is given in Table I. The individual variation in different parts of fruits from the same tree is shown in Table II. The bulbs vary in size, being 1-2½ inches in length and ¼ to 1½ inches in width. Their average weight is 36·9±4·9 grams. The chemical analysis of the different parts of the ripe jack fruit and of the edible portion of green jack fruit is given in Table III. On an average, the edible portion constituted about 46·9 per cent of the whole green fruit, the range being 22·7 to 59·4 per cent.

TABLE I

Composition of ripe jack fruit

Quantity	Weight of a single fruit (lb.)	Bulbs (per cent)	Seeds (per cent)	Rind, core and sheaths covering the seeds (per cent)
Minimum	10·0	15·2	4·1	39·6
Maximum	58·0	49·8	15·9	75·5
Average	18·8	28·7	11·5	59·1

TABLE II

Percentage composition of ripe jack fruit from a single tree

Bulbs	Rind	Core	Seeds	Sheaths covering seeds	No. of seeds per fruit	No. of seeds per 100 lb. fruit	Weight of 100 seeds (oz.)
40·1 ± 1·2	37·8 ± 0·45	5·1 ± 0·26	10·1 ± 0·61	6·7 ± 0·57	148 ± 9·5	703 ± 45	25 ± 1·1

TABLE III—*Chemical composition of ripe and raw jack fruit (moisture free basis)*

Percentage constituent	Bulbs	Seeds	Inner skin (fluffy portion)	Rind (Spiny and fluffy portion)	Raw jack fruit (edible portion)
1. Ash	3·57	3·09	6·49	6·21	6·49
2. Acid insoluble ash	0·15	0·30	0·52	1·92	0·49
3. Ether extract	0·22	1·19	7·46	11·35	7·45
4. Protein (N×6·25)	1·60	12·35	10·51	9·49	10·51
5. Total titratable acidity (as per cent anhydrous citric)	1·24	0·90	1·37	1·24	1·27
6. Crude fibre	3·24	4·44	27·64	30·70	27·64
7. Reducing sugars (as invert sugar)	18·30	0·63	1·70	8·05	1·07
8. Total sugars (as invert sugar)	75·04	0·95	1·91	8·64	1·11
9. Alcohol insoluble solids	15·97	96·58	78·23	..	87·36
10. Starch	..	57·09	9·15	6·24	18·74
11. Carbohydrates other than sugars and starch	15·09	19·99	35·47	26·13	26·79
12. Calcium (mg.)	121·40	379·50	769·30	964·70	881·40
13. Phosphorus (mg.)	68·60	178·20	92·87	90·42	219·50
14. Ferric iron (mg.)	5·13	6·39	12·37	21·63	15·62
15. Ascorbic acid (mg.)	7·78	..	..	..	..
16. Thiamine (Y/100 gm.)	..	221·7	..	..	..
17. Carotene (μy/100 gm.)	16·22	..	..	..	..
18. Pectin (as calcium pectate)	..	..	..	2·62	..
Moisture in the fresh material	72·51	77·03	76·65	79·23	84·17

Discussion

The bulbs and seeds together form on an average about 40·2 per cent of the fruit, with a wide range of 19·3 to 65·7 per cent. Since there are no well defined varieties of jack fruit, it is rather difficult to judge the quality and quantity of bulbs inside from external appearance only. With experience, however, one can with a fair accuracy select good fruit from the colour of the outer skin, the size of spikes and the uniformity of surface of the fruit. Fruits with a uniform surface and with broad and shallow spikes are generally of good quality and contain comparatively more bulbs. Fruits from certain regions like Panruthi in Arcot district of Madras State, are generally of high quality.

On an average, the bulbs, seeds and rind constitute 28·7, 11·5 and 59·1 per cent of the fruit respectively. *Wealth of India* [1948] gives 5·1 as the percentage of seeds in the fruit which seems to be rather low. This may have been probably based on smaller number of observations. The core forms 5·1 per cent and the sheaths of the seeds 6·7 per cent of the weight of the fruit. The rind is generally made of about equal weights of the outer spiny portion and the inner fluffy portions.

The bulbs are rich in carbohydrates. According to Siddappa and Bhatia [1954] these are mostly sugars, namely, sucrose, glucose and fructose. Srivastava [1953] has, however, reported the presence of maltose also. The bulbs contain a fair amount of carotene, but only small amount of vitamin C. They contain protein, fat, calcium, phosphorus and iron in normal quantities like other fruits. The seeds are mostly starchy and contain fair amounts of protein, calcium and thiamine. The inner skin and rind and also the raw jack fruit are mostly fibrous materials, but are fairly rich in calcium. The high values for their ether extract are due to gummy and resinous materials. Fair amounts of protein and starch present are contributed by the rudimentary and undeveloped seeds present in them. The rind is a good source of pectin and can be utilized for the preparation of pectin, pectin extracts and fruit jellies [Siddappa and Bhatia, 1954]. The water extract of the rind contains complex polysaccharides which move slowly on the chromatogrum [Siddappa and Bhatia, 1954].

Summary

On an average, in ripe jack fruit, the bulbs, seeds and rind form 29, 12 and 59 per cent of the bulk respectively. The bulbs are rich in sugars, mostly sucrose, glucose and fructose ; contain a fair amount of carotene but are poor in vitamin C. They contain protein, fat, calcium, phosphorus and iron in quantities which are normally present in other fruits. The seeds, which are mostly starchy, contain fair amounts of protein, calcium and thiamine. The rind of the ripe fruit and the edible portion of the raw fruit, which are mostly fibrous materials, are fairly rich in calcium and pectin. Jack fruit is thus essentially a carbohydrate material and therefore, useful as a source of energy when consumed.

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REVIEW

THE FRUITS, THE SEED AND THE SOIL

EDITED BY W. J. C. LAWRENCE

(*Published by Messrs. MacMillan & Co., London, 1954, pp. 93, Price 5s.*)

THIS is a welcome publication giving in a handy form the information published from time to time by the staff of the John Innes Horticultural Institution, Bayfordbury, Hertford, England, in the form of leaflets.

The topics treated are by no means many but the information on each topic is definitely of considerable practical utility in increasing fruit production. The grower, the gardener and the seedman would all be benefited by following the instructions advocated in this publication (S.S.).

ERRATA

The Indian Journal of Agricultural Science, Vol. XXV, Part II, June 1955

Page	*Line*	*For*	*Read*
143	9	Holmgr	Holmgr.
143	18	*Trop., Agric.*	*Trop. Agric.,*
143	20	*Ceyion*	*Ceylon*
143	28	*Desneux*	Desneux
144	26	Foveolae	foveolae
145	Fig. 1 (explanation) line 4	dorsal	ventral
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Reference to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, title of the article, the abbreviated title of the publication, volume and page. In the text, the reference should be indicated by the author's name, followed by the year of publication enclosed in brackets; when the author's name occurs in the text, the year of publication only need be given in brackets. If the reference is made to several articles published by one author in a single year these should be numbered in sequence and the number quoted after year both in the text and the collected references.

If a paper has not been seen in original it is safe to state 'original not seen'. Sources of information should be specifically acknowledged.

As the *format* of the journal has been standardized, the size adopted being crown-quarto (about 7½ in. × 9⅝ in. cut) no text figure, when printed, should exceed 4¼ in. × 5 in. Figures for plates should be so planned as to fill a crown-quarto page, the maximum space available for figures being 5½ in. × 8 in. exclusive of that for letter press printing.

Copies of detailed instructions can be had from the Secretary, Indian Council of Agricultural Research, New Delhi.